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THE SEPTIC SYPHILODERMATA*

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The purpose of this paper is to show, by reference to the literature, and by citing modern advances in the microbiology, immunology and histopathology of syphilis, that the term "yaws" is simply a 17th century concept of *one* type of syphilide common in all communities having a high incidence of syphilis and a "low index of personal hygiene."

We of the present are at a loss to picture the state of affairs which existed say two or three centuries ago, when even the leaders of society and the rulers of nations might be wig-wearing luetics who rarely took a bath. We doctors have short memories; we recall only the high spots in our interesting historical background! It will encourage better orientation if occasionally we glance through the best works of one, two and three centuries back. By such means not only may we put ourselves en rapport with the most advanced thought of the period in question but we may be surprised to find how much medical medievalism is still in effect in the practice of our profession. In this connection, I would suggest, for surgeons, Samuel Cooper's, A Dictionary of Surgery, 6th Edition, 1830;2 for pathologists, S. D. Gross' Elements of Pathologic Anatomy, 1839;3 for internists, Robert Thomas' The Modern Practice of Physic of 1816; for dermatologists: A Treatise of Diseases Incident to the Skin, 5th Edition, by Daniel Turner, 1736; for the family physician⁵ the works of Dr. Thomas Sydenham, The Third Edition, Revised, by John Swan, M.D., 1753; and for specialists in venereal diseases. Jean Astruc's A Treatise of the Venereal Disease published in 1736.

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Read by invitation at the Seventeenth Annual Meeting of the American Society of Clinical Pathologists, June 9-11, 1938, San Francisco, California.

From the writings of Theodoric, who died in 1298, we learn that mercury was employed in medicine and surgery as early as the 13th century. Its use in venereal diseases was first mentioned in a tract by Juan Almenar, published in 1516. The ancients considered mercury a poison and we know they did not use it. The good effects which accompanied the use of mercury in cutaneous affections such as itch, eruptions and ulcers first led to its use in venereal cases. Paracelsus (1493–1541) first showed that mercury might be given internally with safety. Before this it had been used externally only in the form of ointments, liniments and plasters, and by fumigation.

Ancient peoples therefore had no specifics for syphilis; and as we are now well aware of the ancient European ancestry of syphilis from the writings of many scholars in France, Germany, Italy, Great Britain and America, we should give thought to

what happened to their syphilitic infections.

Wherever syphilis is not treated generation by generation with effective specifics, it eventually affects the entire population: not as a venereal disease, but as one of the exanthemata of childhood. This type of syphilis is that which infects most of the dark-skinned races of the tropics today. It is this which E. Herndon Hudson has described so comprehensively for the Bedouins of Mesopotamia. It is the syphilis of Central China and India and was the type so prevalent in Bible times. For when syphilis is not specifically treated, eventually the virus and the tissue-cell come to an understanding, so to speak, the disease then becoming latent in most of the adult population. A few individuals are cured by unaided nature, a few come down with the osseous and mutilating lesions, but the vast mass of population remains symptom-free throughout life in spite of showing a Wassermann incidence of over 80 per cent. Saddle noses and other evidence of congenitalism may be present; but, and this is one of the peculiarities of the bejel and framboesial types of syphilis, very few persons infected with either of these types ever show the nervous and vascular accidents so common among enlightened races.

After specific qualities were established for mercury, physicians

came to use it in such excessive quantities that they almost caused its abandonment as a remedy. For this reason the sixteenth and seventeenth centuries were critical ones as regards the treatment of syphilis. By 1830 there was a school of physicians who believed that systemic syphilis was better left alone and the disease treated for its local lesions and by means of tonics. When mercury was administered by this group, it was given in small amounts and not long continued. Towards the end of the 19th century, however, there came about a better understanding of the way mercury acts, how it is eliminated and how best to administer it. During the 38 years of the 20th century. arsenicals, bismuth and other specifics have been introduced, and the treatment of syphilis so revolutionized that we are now definitely on the road to eradication of this disease although actually a long way from that goal. It is now evident, however, that the incidence of syphilis in any race constitutes an infallible index of the state of social advancement of that people.

From this brief survey we may draw lessons which will enable us to define the problem of what is necessary in order to eradicate syphilis from the world. One of the accomplishments of our medical friends of the "American-origin fantasy" is that they have so befuddled the profession as to proper procedure that, if their views prevail, the world will never be free of syphilis. They would start "eradicating" at the top instead of the bottom of the heap of Homo sapiens syphiliticus!

From the treatment standpoint we may divide syphilis into three periods, and these periods are running concurrently in different races of people on the earth today. We have in yaws and bejel the biblical type of syphilis in which no specifics were used. In some backward populations, specifics are still used to excess, while with the most cultured races, syphilis is properly treated and is definitely on its way out. When no treatment is given the whole population is tainted. When the population is too intensively treated the individuals are deprived of the benefits of the immunity which develops when treatment is withheld. When specifics are used we have sequelae develop which are mostly absent when the disease is allowed to run its whole course

without treatment. These questions will be worked out so that we may definitely establish the best practice under all circumstances. This will be delayed however until we get over the folly of trying to make a separate disease out of yaws and a third one out of bejel!

From this brief survey it will be seen that in some of our concepts about syphilis we are centuries behind. In many, which particularly holds true for yaws and bejel, we are 100 years behind. In some features our practice is abreast of the best in modern medical thought, while in a few we have allowed scientists in medicine to flatter us into the fatal error of thinking that pure research is the only kind of science that is worthy. If we had meditated more, and "researched" less on syphilis during the past century, if we had digested our findings in the microbiology, immunology, and histopathology of syphilis, we should long ago have unhorsed those professional brothers who would have us believe that the "honey colored crust" which they describe as the sine qua non of their distinct disease, "yaws," is anything but the dried secretion from a badly contaminated pustular syphilide, in which the body is putting forth all and every force at its disposal in order to cure the unfortunate wretch of his mixed microbic infections.

Perhaps the simplest classification of syphilodermata ever devised is that given by Unna in his Pathology of Diseases of the Skin, published in 1894:

- I. The Simple papular syphilides.
 - (a) The small papular syphilide;
 - (b) The large papular syphilide and its varieties:
 - (a) The condylomatous papule,
 - (β) The palmar papule,
 - (γ) The bullous papule of infants; and in conclusion,
 - (c) The healing of the syphilitic papule.
- II. The mixed papular syphilides.
 - (a) The papulo-crusted syphilide,
 - (b) The papulo-crusto-pustular syphilide (Ecthyma),
 - (c) The varicelliform syphilide,
 - (d) The varioliform syphilide.

The organism (*T. pallidum*) causes pretty much the same reaction in the tissues at all stages; i.e., it acts as a slow irritant, and the tissue response is chiefly on the part of the non-granular leucocytes and connective tissue cells, there being as a rule practically no chemotactic effect on the polymorphonuclear leucocytes.

The gumma of the tertiary stage is a cellular granulation tissue mass, which at first may present a pale pinkish and somewhat translucent appearance; but the central parts soon undergo necrosis, and accordingly it usually consists of vellowish and necrotic material, surrounded by fibrous tissue. In course of time gummata have a great tendency to undergo absorption and shrinking, and thus cicatricial areas are a common result. In addition, chronic interstitial fibrosis, often of a spreading character, is a common lesion, sometimes associated with distinct gummata, sometimes apart from them, and this chronic inflammation may be attended at places by necrosis or gummatous Structurally a gumma resembles closely the primary sore, but differs in the occurrence of necrosis at an early period. due in part to obliterative changes in blood vessels, and in part to direct action of treponemes, although they are present only in small numbers. Such explanation of the necrosis is unsatisfactory and it is justifiable to regard the process as chiefly due to increased tissue sensitiveness or to allergy developed in the course of the infection. The necrotic areas may preserve cellular outlines for a considerable time, these not having the tendency to fuse into amorphous material which is observed in tuberculosis. Giant cells may be present in granulation tissue at the periphery, but are usually smaller than in tuberculosis and there is an absence of concentric formation of endothelioid cells around them, resulting in follicles. Nevertheless differential diagnosis may be a matter of difficulty, as tuberculosis may occur without characteristic features. Important vascular lesions in syphilis are due to lodging of treponemes in the adventitial sheath, whence they extend into the media. They give rise to cellular infiltrations in these coats of the nature described, and sometimes necrosis may follow.

Congenital syphilis is histologically similar but presents special features because of large numbers of treponemes often present. Thus diffuse proliferations of connective tissue cells occur in liver, lungs, pancreas etc. and there may be also minute foci of more intense reaction followed by necrosis (miliary gummata). Such lesions may occur throughout the affected organs and lead to death at an early period or they may be more localized and be followed by fibrotic change. Over 100 years ago, Samuel Cooper wrote:

I firmly believe, that, with respect to all the appearances of this disease, a vast deal depends upon constitution, independently of the nature of the virus. And I adopt this opinion, at the same time that many reflections already hinted at in this article lead me to join in the belief, that syphilitic diseases may depend upon a variety of poisons, whereby some of the perplexity of these cases may be explained.

Most of Unna's "syphilides" both early and late are in fact mixed infections. His error is a little less evident to us perhaps than Hunter's folly of classifying all venereal infections as due to one virus. Pathologically, however, his grouping is as far from the facts as that gonorrhoea and syphilis are the same disease. Fortunately the usual complications are pyogenic and the concomitant local polynucleosis shows the basic fact to the pathologist because he knows that the only pure treponematous eruptions are the roseolas and perhaps the papular syphilides, both of which are histologically pure plasmomata. Wherever there is a movement of polynuclear cells into or above the plasmoma, we are dealing with a mixed infection. Now the gumma. if uncomplicated, will undergo necrosis from shutting off nourishment to its center as stated. When pus infection occurs, then we are likely to have loss of tissue and scarring, although these events, as implied, may occur without ulceration.

The same is true of the early pus-infected lesions such as condylomatous, framboesiform and circinate types of eruption. All of Unna's mixed papular syphilides are, therefore, mixed infections, for *Treponema pallidum* never calls out the polynuclear leucocyte and never liquefies the tissue cell.

The unscientific practice of trying to draw conclusions as to

duality of viruses as between yaws and syphilis from animal inoculations of the mixed antigens found in highly septic yaws lesions has been pointed out by me⁸ and is here again especially stressed. In this connection I wish also to call the attention of the American Society of Clinical Pathologists to the most unsatisfactory diagnostic evidence upon which several strains of Treponema pertenue have masqueraded as valid species.

(1) The strain developed by Nichols⁹ in 1910 finally "acted up" in such a disgracefully unframboesial way that Nichols

himself had to throw this type species overboard.10

(2) The strain secured by Jav F. Schamberg and Joseph V. Klauder from an American soldier in France¹¹ who had never been where yaws is prevalent, or associated with anyone who had yaws. Here is the way they made their "Louis Quinze period" diagnosis upon him. Dr. Castellani himself told them what to do in these words: "From the description you give and the photos, I would diagnose the case as one of yaws. I am not aware of the disease having been previously recorded in France. but of course during the war a large number of native troops and native workmen were imported into France from tropical countries, and they may have imported the disease with them. As regards mode of infection I am inclined to give more importance to direct contact than to insect carriers. Of course, in the tropics there is no doubt that in many cases the disease is carried by flies." Such "remote control" in so important a matter as the diagnosis of a condition from which a new species of organism was isolated and minutely differentiated from Treponema pallidum is, I think you will agree, not impressive.

(3) Lastly the strain secured by Howard Fox,¹² from a West-Indian negress who turned up in New York with a corymbiform type of eruption upon her. Drs. Wade H. Brown and Louise Pearce having obtained a treponeme from the lesions proceeded to study the eruption, making the following comment: "The conditions simulated are tuberculosis verrucosa, syphilis, blastomycosis, sporotrichosis and kerion. Tuberculosis verrucosa is excluded by lack of giant cells and the large number of plasma cells. Syphilis can be less readily excluded but gummas prac-

tically never show the verrucoid appearance here exhibited and the vascular changes are on the whole not sufficiently profound. The other three conditions are excluded by the relatively small number of true abscesses. By exclusion, diagnosis remains: Yaws."

In an article entitled Comparative Histology of Yaws and Syphilis in Jamaica¹³ Ferris and Turner publish an excellent and well-documented paper giving many fine illustrations showing the histology of these two conditions. There is nothing in this long paper, however, which would permit the most pronounced partisan of duality to find any differences which an enquiring pathologist, seeking for truth, could depend upon. To my mind this is the final proof that yaws is just one of the septic or infected types of syphilide comparable in every respect with Unna's condylomatous papule and his papulo-crusted syphilide.

CONCLUSIONS

1. Races of man not treating syphilis with specifics develop a type of this disease which in many respects differs from venereal syphilis.

2. The differences are evidenced in epidemiology, pathology, symptomatology, immunology and in the absence of the serious vascular and nervous sequelae following inadequate and ill-timed specific treatment.

3. It is pointed out that several strains of Treponema pertenue, so-called, have been determined upon inadequate evidence.

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HISTORY OF SYPHILIS IN 180 PATIENTS IN WHICH THE KLINE TESTS, THE KOLMER TEST AND THE KAHN TEST ARE IN DISAGREEMENT*

JOHN H. MILLS AND ELSA JAHN

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The University Hospital subjects all blood specimens to the Kline exclusion test for syphilis. Specimens which show any degree of reaction to this test are subjected to the Kline diagnostic test, the Kolmer and the Kahn tests. Of 10,610 blood specimens examined 4,785 were examined by all four tests. Of these 4,785 specimens the results were in disagreement in 180 patients.

The histories of these patients were examined to determine the presence or absence of syphilitic infection. The results are

presented in the accompanying table.

It may be seen that 45 per cent of these 180 patients unquestionably had been infected with syphilis. The history was strongly suggestive of a syphilitic infection in an additional seven per cent. In the history of the remaining 48 per cent either no mention was made regarding the presence or absence of syphilis, or the infection was denied. Since the histories in many instances were extremely meager, and since about half of the patients studied were negroes in whom the morbidity rate for syphilis is high in Baltimore, many patients included in the group that did not give a history of infection actually suffered from the disease.

As may be seen from table 1 the results of the tests do not follow a definite pattern such that the probable presence of infection might be deduced from a certain type of disagreement between tests. However the probable presence of many actually infected persons in the group giving no history of syphilis may obscure such a pattern.

^{*}Received for publication October 5th, 1938.

It has not been routine practice to subject specimens of blood which are negative to the Kline exclusion test to other serological tests for syphilis, but in one instance where this was done a strongly positive Kolmer test was obtained. The negative Kline exclusion test and the strongly positive Kolmer test were verified

 ${\bf TABLE~1}$ Results of Conflicting Serological Tests for Syphilis in 180 Patients

ELINE EXCLU- SION	KLINE DIAG- NOSTIC	KOLMER KAHN		NO HISTORY OF SYPHI- LIS	HIS- TORY OF SYPHI- LIS	HIS- TORY DOUBT- FUL FOR SYPHI- LIS
Total				86	82	12
Negative	Negative	Doubtful	Doubtful	0	1	0
Doubtful			4	5	0	
Doubtful	Doubtful	Negative	Negative	20	11	3
Doubtful	Doubtful	Negative	Doubtful	11	6	2
Doubtful				3	0	0
Doubtful Negative Doubtful Doubtf		Doubtful	2	1	0	
Doubtful Negative		Doubtful Negative		4	18	1
Doubtful	Doubtful	Negative	Positive	0	2	0
Doubtful	Doubtful	Positive	Negative	0	3	0
Doubtful	Negative	Positive	Negative	6	15	0
Doubtful	Negative	Positive	Doubtful	0	2	0
Positive	Doubtful	Negative	Negative	29	15	7
Positive	Doubtful	Anticomplementary	Negative	2	3	0
Positive	Doubtful	Anticomplementary	Doubtful	0	1	1
Positive	Doubtful	Negative	Doubtful	8	4	0
Positive	Doubtful	Positive	Negative	0	1	0
Positive	Doubtful	Negative	Positive	1	0	0
Positive	Positive	Doubtful	Negative	0	1	0
Positive	Positive	Doubtful	Positive	0	1	0
Positive	Positive	Negative	Negative	3	0	0
Positive	Doubtful	Doubtful	Negative	2	2	0
Positive	Negative	Doubtful	Negative	2	2	0

by repeat tests on the same blood specimen. It was not possible to ascertain whether this patient had a history of syphilis.

Blood specimens from five hundred individuals which yielded negative results to the Kline exclusion test were subjected to the Eagle complement fixation test. The blood specimens from two individuals gave doubtful results, which were definitely negative on repeat testing of the same specimen. In one instance the same blood specimen gave doubtful results in repeat tests to the Eagle complement fixation test and negative results to the Kline exclusion test. The patient furnishing this specimen had been treated for a fracture and a medical history was not obtained. Follow up investigation was not possible.

These results suggest that syphilis is present in half or more of the patients in whom doubtful and disagreeing reactions are obtained in multiple serological tests on the same specimen. No single test gives as satisfactory results as a combination of tests preferably employing both complement fixation and flocculation procedures.

The Kline exclusion test is not a screen test in the sense that it excludes all blood specimens which give positive reactions to other satisfactorily specific serological tests.

A SIMPLIFIED COMPLEMENT FIXATION TECHNIC FOR THE SEROLOGIC DIAGNOSIS OF SYPHILIS*

FRED BOERNER AND MARGUERITE LUKENS

From the Laboratories of the Graduate Hospital, University of Pennsylvania, Philadelphia, Pennsylvania

The venereal disease control campaign now being waged under the leadership of the United States Public Health Service, is producing a constantly increasing demand for laboratory studies in the diagnosis of syphilis. Such work requires technical skill which can be acquired only after careful training under experienced teachers and by persons who have a natural aptitude. Even then the reliability of their results can never be taken for granted. Obviously, it is during the latter part of the training period that there is greatest need for a means by which technical accuracy may be checked at unannounced intervals, and there is always required a means by which each step in the procedure may be checked by the technician himself. In the usual set-up of the routine complement fixation test there are measurements and manipulations which cannot be adequately inspected by intermittent supervision. Furthermore, the experienced worker himself cannot, by merely glancing at the various tubes in the rack, always confirm his belief that every measurement has been made in exactly the right way.

The exquisite accuracy in diagnosis which is the aim of elaborate quantitative tests is a laudable ambition. Experience through several years in the training of technicians has brought the conviction that the construction of a test which will assure routine technical accuracy is at least equally important.

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When time and cost are opposed to accuracy in the evaluation of any laboratory procedure the decision is never in doubt. But when economy and accuracy can be combined in a modified technic, there is an additional, however minor, point in its favor. In a laboratory where thousands of tests a year are made, the item of the cost of reagents cannot be ignored.

A technical procedure is described herein which actually has the qualities of reliability combined with speed, economy, and especially that most desirable of all essentials, it can be checked at every stage and step, accurately and completely, by the person supervising the work, and by the technician himself.

The main differences which apply to this procedure lie in the mixing of certain of the reagents in bulk. Antigen and complement are combined and added as a single increment to the patient's serum, and finally sheep erythrocytes are sensitized and measured thus into the various tubes at one time and not as two different additions. In the actual performance of the test, therefore, the serum of each of the patients is placed in the tubes provided for them, the rack is held up and inspected, and an omission or an excess is readily detected. The antigen and complement have already been titrated and mixed in the proper proportions, in bulk: the specified, unvarying, quantity of the mixture is measured into each tube; the rack is held up and inspected; and the tubes are placed in the ice box to permit fixation. At the proper time, after removal from the refrigerator, the suspension of previously sensitized erythrocytes is added. a fixed amount to each tube; then the contents of the rack are inspected; again omissions and excesses will be conspicuous; the rack is shaken and placed in the water bath; and the results of the test are read in the usual manner.

The advantages, in the direction of increased accuracy, of mixing the reagents in bulk are so obvious they scarcely need to be mentioned. The great saving of time would be a negligible quantity were it not combined as a kind of by-product, with greater accuracy.

As will be noted in a subsequent publication, experience in making tests by this technic has amply demonstrated its re-

liability. This work has been controlled by parallel tests made in the Serology Laboratory of the Graduate Hospital, using the Kolmer complement fixation and the Kahn and the Eagle flocculation tests.

THE REAGENTS

Sheep erythrocyte suspension: Prepare a 1.5 per cent suspension of washed sheep corpuscles in 0.85 per cent saline. We advise keeping the blood in the refrigerator for at least 48 hours before washing the cells.

Hemolysin: Anti-sheep hemolysin is used. The rabbit serum is preserved with an equal volume of neutral glycerine. For convenience, a 1:20 stock solution is prepared as follows:

	cc.
Glycerine-hemolysin (50 per cent)	2.0
Saline solution	
Glycerine C. P.	9.0

Complement: Fresh pooled guinea pig serum (at least five pigs), is recommended. For use in the test, a 1:30 dilution is prepared.

For those who do not need large amounts of complement, the procedure of Sonnenscheine¹ for the preservation of complement is recommended. It has been shown by Green² to be a most satisfactory method for this purpose. As modified and used in our laboratory, it is as follows:

1. Bleed several guinea pigs and allow the blood to clot.

2. Separate the serum and add an equal volume of the following mixture:

Sodium acetate	12.0 grams
Sterile distilled water q.s.	

3. Store in the freezing unit of the ice box and allow it to remain frozen until it is needed.

It is claimed that there is little or no loss of activity even after 7 to 12 months storage at 4°C. Our own observations do not extend beyond 8 weeks. During this period the results have been entirely satisfactory.

Another satisfactory method for preserving complement is that of drying it in the frozen state in vacuo by either the lyophile or cryochem process.^{3, 4} We have had perfect results with complement preserved for a year by the lyophile, and for more than a year by the cryochem process.^{5, 6}

Both Eagle^{4a} and Kolmer^{4b} have reported satisfactory results with lyophile complement from 10–13 months old.

Antigen: The antigen recommended for use in this test is a cholesterolized ether-alcohol extract of powdered beef heart (Difco). The method of preparation was developed by the authors in collaboration with Dr. Charles A. Jones.⁹

Details regarding its sensitivity and specificity will be published separately.

The following is the method for its preparation.

- 1. Place 10 grams of powdered beef heart (Difco) in a flask of 1 L or 2 L capacity.
- 2. Add 225 cc. of absolute alcohol and 75 cc. of ether (U.S.P.); and allow to stand at room temperature for ½ hour, shaking thoroughly every 5 minutes.
 - 3. Filter through filter paper and discard the residue.
- 4. Place the filtrate in a large flask or beaker and evaporate it, using slight negative pressure, to 50 cc. by boiling in a water bath. If the concentrated extract is less than 50 cc., add sufficient absolute alcohol to make it up to this amount.
- 5. Place the concentrated filtrate in the refrigerator for 1 hour. A heavy precipitate will form.
- 6. Filter through paper. This filtration should be done in the refrigerator so that the filtration will be completed while the solution is still cold.
- 7. Weigh 200 mgm. of cholesterol and dissolve it in the 50 cc. of filtrate. This is the finished antigen, and it is now ready to be tested for its optimum fixing dose and its anti-complementary properties.

Note: If it seems desirable that the antigen be more or less sensitive, it is necessary merely to vary its cholesterol content. Antigens prepared by the above method are slightly more anti-complementary than the alcohol used in their preparation, and their optimum antigenic dose is usually between 1:800 to 1:1,600. Any volume of antigen can be prepared by this method by proportionally increasing or decreasing the amounts given.

TITRATION OF ANTIGEN FOR ANTI-COMPLEMENTARY PROPERTIES

- 1. Arrange 6 tubes in a series and mark them 1 to 6.
- 2. Add 0.5 cc. of 1:30 dilution of complement to each tube.
- 3. Prepare a 1:10 dilution of antigen in saline solution (0.2 cc. of antigen to 1.8 cc. of saline).
 - 4. To each tube add the amount of antigen indicated in table 1.
- 5. Incubate at 8 to 10°C. in the refrigerator for 15 to 18 hours, then for 10 minutes at 37°C. in a water bath.
- Add 0.5 cc. of sensitized cells prepared in the manner recommended for the test itself.
- 7. The smallest amount of antigen showing the slightest degree of inhibition of hemolysis is the anti-complementary dose.

TITRATION OF ANTIGEN FOR ANTIGENIC PROPERTIES

The following method for determining the optimum dose of antigen is that first described by Hooker⁵ and since studied and recommended for routine use by the authors.⁵

Our work has also been confirmed by Kolmer^{6a} and he has adopted the method for his test.

- 1. Arrange 4 rows of tubes, 6 tubes in each row.
- 2. Add 0.5 cc. of complement (1:30) to all tubes except the first and second of each row.
- 3. Prepare a 1:400 and a 1:600 dilution of antigen in complement (1:30) as follows: Add 0.1 cc. of antigen to 0.9 cc. of complement solution (1:30); dilute 0.3 cc. of this 1:10 dilution with 11.7 cc. of complement solution. This makes a 1:400 dilution of antigen. Dilute 6 cc. of this 1:400 solution with 3 3 cc. of complement. This makes a 1:600 dilution of antigen.

TABLE 1

TUBE	COMPLEMENT (1:30)	ANTIGEN 1:10		CORPUSCLES 0.75 PER CENT	
	cc.	cc.		cc.	
1	0.5	0.5	Incubate 8	0.5	Incubate at
2	0.5	0.4	to 10°C. for	0.5	37°C. for
3	0.5	0.3	18 hours,	0.5	1 hour
4	0.5	0.2	10 minutes	0.5	
5	0.5	0.1	at 37°C.	0.5	
6	0.5	0.05		0.5	

TABLE 2
An Example of Titration of Antigen

SERUM	ANTIGEN DILUTIONS					
SMACM	1:400	1:600	1:800	1:1200	1:1600	1:2400
cc.						
0.0125	_	±	4	4	4	1
0.025	2	3	4	4	4	2
0.05	4	4	4	4	4	4
0.1	4	4	4	4	4	4

- 4. Add 0.5 cc. of the 1:400 dilution of antigen to the first and third tube of each row.
- 5. Mix the contents of the third tube in each row and transfer 0.5 cc. to the fifth tube and then discard 0.5 cc. from this tube.
- 6. Add 0.5 cc. of the 1:600 dilution of antigen to the second and fourth tubes in each row.
- 7. Mix the contents of the fourth tube in each row and transfer 0.5 cc. to the sixth tube and discard 0.5 cc. from this tube.
- 8. To all tubes of the first row, add 0.1 cc. of inactivated positive serum. To all tubes of the second row, add 0.1 cc. of positive serum diluted 1:2 in saline.

To all tubes of the third row, add 0.1 cc. diluted 1:4. To all tubes of the fourth row, add 0.1 cc. diluted 1:8.

- 9. Mix all tubes by gentle shaking and incubate in refrigerator at 8 to 10° C. for 15 to 18 hours, followed by 10 minutes at 37° C.
 - 10. Add 0.5 cc. of 0.75 per cent suspension of sensitized cells.
 - 11. Incubate at 37°C. for 1 hour.
- 12. Record the reaction obtained in each tube. The dilution of antigen that gives the strongest reaction with the smallest amount of serum is taken as the optimum dose. This is the dose which will be used in the test.

Note: Antigen titrations do not have to be made for each test. Change in titer is very slow. It is safe to repeat the titration only once in 3 or 4 months.

As noted in paragraph 12 (above), the optimum dose of an antigen giving the results noted in this tabulation would be somewhere between 1:800 and 1:1,600. Therefore, we would probably use of this antigen, a dilution of 1:1,200.

TABLE 3

TUBE	1:75	HEMOLYSIN HEMOLYSIN DILUTION
	cc.	
1	0.9	0.1 cc. of 1:100 = 1:1,000
2	0.8	0.2 cc. from tube 1 = 1.5,000
3	1.3	0.2 cc. from tube 1 = 1.7,500
4	0.5	0.5 cc. from tube 2 = 1:10,000
5	0.5	0.5 cc. from tube 3 = 1:15,000
6	0.5	0.5 cc. from tube 4 = 1:20,000
7	0.5	0.5 cc. from tube 5 = 1:30,000
8	0.5	0.5 cc. from tube 6 = 1:40,000

ADJUSTING THE HEMOLYTIC SYSTEM

- 1. Prepare a 1:75 dilution of complement by diluting 4 cc. of the 1:30 dilution, which is prepared for use in the test itself, with 6 cc. of saline solution.
 - 2. Arrange 8 tubes in a series and mark them 1 to 8 inclusive.
- 3. Prepare 1:100 dilution of hemolysin in complement by adding 0.1 cc. of the 1:20 stock solution to 0.4 cc. of 1:75 complement, and make further dilutions as shown in table 3.

Discard 0.5 cc. from tubes 3, 7, and 8.

- 4. To all tubes, add 0.5 cc. of a 0.75 per cent corpuscle suspension (1.5 per cent suspension plus an equal volume of saline).
- Mix by gentle shaking and place in a water bath at 37°C. for 30 minutes.
 - 6. Note the highest dilution of hemolysin which gives complete hemolysis.
- 7. For sensitizing the corpuscles to be used in the test proper, four times this amount is used. When the hemolysin and cells are mixed, the amount

actually used in the test is twice the smallest amount found to give complete hemolysis in the above titration, e.g., if the smallest dilution giving complete hemolysis is 1:40,000, then a dilution of 1:10,000 is prepared, and this is mixed with an equal volume of a 1.5 per cent suspension of corpuscles 15 minutes to 1 hour before the cells are used in the test.

A complement which does not produce complete hemolysis in the 1:5,000 dilution is considered unsatisfactory.

PREPARATION OF ANTIGEN-COMPLEMENT MIXTURE

1. Estimate the amount of antigen-complement mixture desired, allowing 0.5 cc. for each dose. Place this amount of 1:30 complement solution in a flask.

2. Add slowly, with agitation, the amount of antigen (1:10), required to make the desired dilution, as determined by preliminary titration as described above.

Example: If the amount of antigen desired for the test is 100 cc. of a 1:800 dilution, then 0.125 cc. of antigen is dropped into 1.125 cc. of 1:30 complement. This 1:10 dilution of antigen is then added to 100 cc. of 1:30 complement. This is the antigen-complement mixture and can be labeled A-C mixture.

A simple method for calculating the amount of 1:10 antigen to be prepared for addition to any given amount of complement is as follows:

Let: X = the amount of 1:10 antigen to be added to the 1:30 complement.

A = the amount of antigen-complement mixture to be prepared.

B= the desired dilution of antigen (determined by previous titration). Then:

$$\frac{A}{B} \times 10 = X$$

e g.: To prepare 150 cc. of A–C mixture, carrying a 1:1,200 dilution of antigen, $\frac{150}{1,200} \times 10 = 1.25$ cc. of 1:10 dilution of antigen is added to 150 cc. of 1:30 complement.

COMPLEMENT FIXATION TEST

This technic can be used for either a qualitative or a quantitative test. For the qualitative test only one dose of serum is employed. For the quantitative test additional doses of serum are used.

Qualitative test

1. For each serum to be tested, 2 tubes are set up. To both tubes add 0.1 cc. of serum. Place the tubes in water bath at 58°C. for 10 minutes for the purpose of inactivating. In addition, the controls in table 1 are included.

2. To tube 1, add 0.5 cc. of antigen-complement mixture (antigen diluted in 1:30 complement), so 0.5 cc. contains proper dose of antigen.

3. To tube 2, add 0.5 cc. of complement (1:30).

- 4. Mix all tubes thoroughly and place in refrigerator at 6 to 10°C. for 15 to 18 hours.
 - 5. Place tubes in water bath at 37°C. for 10 minutes.
- 6. To both tubes, add 0.5 cc. of 0.75 per cent sensitized corpuscles. (The corpuscles and hemolysin should be mixed at least 15 minutes before use.)
 - 7. Mix thoroughly and place in water bath at 37°C. for 1 hour.
 - 8. Reactions are interpreted as follows:

$$++++$$
 = Positive
+++, ++, + or \pm = Doubtful
- = Negative

- 9. When several doses of serum are used for the purpose of making a quantitative determination of the degree of positiveness of the serum, the reactions obtained with each dose are recorded for comparison with previous or subsequent tests. For routine work, the American Committee on Evaluation of Sero-diagnostic Tests for Syphilis has recommended reporting reactions only as positive, doubtful, or negative.
 - 10. Anti-complementary sera are reported as follows:

Reaction	Interpretation
4 4	Anti-complementary
4 3	Anti-complementary
4 2	Anti-complementary
3 2	Anti-complementary
4 1	Doubtful
4 ±	Doubtful
3 1	Doubtful
3 ±	Doubtful
$2 \pm$	Doubtful
3 3	Negative
2 2	Negative
2 1	Negative
1 1	Negative
1 ±	Negative
± ±	Negative

A known positive and a known negative serum are run with each test as additional controls.

Quantitative test

- 1. For each serum to be tested, 3 or more tubes are used, depending upon the number of serum dilutions desired.
 - 2. Into all tubes except the last, place 0.2 cc. of saline solution.
 - 3. Add 0.2 cc. of serum to the first tube and 0.1 cc. to the last tube and mix.
 - 4. Transfer 0.2 cc. from the first to the second tube; mix and transfer 0.2 cc.

from the second to the third tube; and continue transfer until the next to the last tube is reached and then discard 0.2 cc. The last tube is the serum control.

- 5. Place the tubes in water bath at 58°C. for 10 minutes.
- 6. Continue in the same manner as described under the qualitative test, starting with step 2.

TEST USING CEREBROSPINAL FLUID

1. The qualitative test is the same as that described for blood serum, except that cerebrospinal fluid is mixed with negative serum and larger amounts used. To both tubes, add 0.25 cc. cerebrospinal fluid and 0.1 cc. of cerebrospinal fluid.

TABLE 4
SIMPLIFIED COMPLEMENT FIXATION TEST (QUALITATIVE)

TUBE	BERUM		A-C* MIXTURE	COMPLEMENT 1:30		GENEITIZED CELLS (0.75 PER CENT)		RESULTS
1 2 Antigen control	0.1 0.1 0.1†	10 min- utes at 58°C.	0.5 0.5	0.5	15-18 hours at 8- 10°C. followed	ec. 0.5 0.5 0.5	1 hour at 37°C.	Hemolysis
Complement control Corpuscle control	0.5††	,		0.5	by 10 minutes at 37°C.	0.5		Hemolysis No hemolysis

^{*} Antigen-complement mixture.

2. The quantitative test is the same as described for blood serum except the cerebrospinal fluid is diluted in the following manner: To all tubes except the first and last, add 0.25 cc. of saline solution; then add 0.25 cc. of cerebrospinal fluid to the first, second, and last; mix and transfer 0.25 cc. from tube 2 to tube 3, continuing transfers until next to the last tube, and discard 0.25 cc. To all tubes add 0.1 cc. of negative serum.

DISCUSSION

The simplified technic for the complement fixation test effects a marked reduction in both cost and labor. It minimizes the

[†] Negative serum.

tt Saline.

chances of error not only be reducing the number of steps, but by the fact that if an error is made, especially the omission of any step, it is readily detected even after the completion of the test.

The small amount of serum required (0.2 cc.), is an advantage, since it leaves sufficient serum for the flocculation tests, or for repeating when desired. Sufficient blood can be obtained by finger puncture, if only the Wassermann test is desired or when venous puncture is inadvisable or difficult.

The omission of a second dose of serum in the qualitative test is justified, we feel, by the fact that, in over 12,000 tests, prozone reactions were so extremely rare that the information obtained was far too small to compensate for the expense and labor incurred. It may be said that the second dose is of value as a check on technic. We have found it of very little value in this respect and practically unnecessary in the simplified technic.

The adjustment of the hemolytic system is made by titrating the hemolysin with less than one-half the dose of complement recommended for use in the test. This insures a dose of complement which is never less than two and one-half units, and a dose of hemolysin which is always at least two units. The practice of diluting the antigen in the complement solution permits the addition of both reagents in one manipulation. The use of sensitized cells not only permits the addition of both hemolysin and cells in one manipulation, but also, from our experience, is a far better practice than adding the two separately and depending upon uniform sensitization by shaking tubes.

The antigen recommended for this test is prepared by a simplified method which will be discussed in a separate publication. It consists of an ether-alcohol extract and contains only substances which are soluble in both alcohol and ether, and therefore differs from alcoholic extracts which contain ether insoluble material.

CONCLUSIONS

1. A simplified complement fixation technic for the diagnosis of syphilis is described.

- 2. This technic reduces time and cost to about one-half that of the tests in common use, without reduction of specificity.
- 3. It reduces the chances of error in measurements through mixing the reagents in bulk.
- 4. It readily permits inspection and checking by a supervisor or by the technician himself of each step in the procedure.
- 5. It is equally applicable to quantitative and qualitative tests.

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TUMOR NOMENCLATURE: SUGGESTIONS FOR ITS REVISION*

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The inherent desire to modify or supplement a classification must and will be critically opposed by all learned societies until the suggested changes or additions have demonstrated their reasonableness and value. On the other hand, the perfectly natural "laissez faire" attitude, which would hold us to the terms and concepts propounded by our elders, is apt to stifle initiative and confine us to the tortuous grooves and ruts of bygone years.

It is with these two "tendencies" in mind that I am urging this society to survey critically the present nomenclature of tumors. I hope that such a survey will not only modernize but simplify our use of "names" and will express in better terms the present day ideas of what the various hyperplasias really represent. In order to provoke discussion I shall emphasize rather radically certain phases of the question and will expect that our more conservative confrères will oppose these suggestions. Ultimately the inevitable compromise may give us a more modern and more rational classification.

The first objective is to examine the content or extent of our ideas as to what constitutes a true tumor. Definitions are almost as numerous and varied as the pathologists who study them. Without reviewing the confusion of these definitions and for simplification I would suggest that when we use the term "tumor" or "newgrowth" or "neoplasia" or "neoplastic disease" we mean "a more or less disorganized and independent hyperplasia of tissues, which tends to invade and metastasize."

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The next step is to abolish completely the terms "malignant" and "benign" as applied to true tumors. Theoretically no neoplastic disease can be designated a "benign" tumor. They are all *inherently malignant*. The old rules supposedly differentiating these two entities and still part of the students' curriculum are no longer necessary or applicable.

Because of long usage and clinical implications and because newgrowths are now often classified according to "degrees" of "malignancy," it is doubtful if we can successfully obliterate these two adjectives, especially if it is planned to retain one to express "degrees" of rapidity of growth. But it would probably be more rational to drop both of them and imply our conception of what constitutes "degrees" of malignancy by simple grading indices like 1, 2, 3, 4, and so forth.

And here it becomes necessary to inquire more closely into our notions about so-called degrees of malignancy. I have previously thought and taught that an increase in "malignancy" was directly in proportion to the resemblance of a newgrowth to an embryonic status. Lack of differentiation was inferred to be an approach in morphology to that of cells in early fetal life. I am now convinced this is a mistake. Differentiation of a tumor occurs in proportion to its rate of growth and is affected somewhat by the "soil" in which it grows. At least all so-called mensurations of so-called malignancy must be based upon the presence or lack of differentiating characters, that is, signs of organization.

Parenthetically it may be added that even if such measurements differ for different parts of the same tumor or in various sites of metastasis or in the judgment of more than one observer, the general scheme of grading, as expressing a notion of the relative menace, rate of growth, destructiveness, or better, differentiation, of a given tumor is a distinctly useful advance in the study of neoplasia.

In any given abnormal mass of tissue the following questions become definitely pertinent:

1. Is it a neoplasm? If not, is it inflammatory, such as a granuloma; or developmental, such as certain cysts; or the result of perverted functional hyperplasias, such as leiomyomas, certain adenomas, and so forth?

2. If it is a neoplasm, where is the original site and what other manifestations of unrestrained growth, such as invasion or metastasis, are present and where?

3. Is it a connective tissue growth? If so, it is a sarcoma and there are various adjectives which are useful in designating its morphologic characteristics, type of growth and differentiation, such as "fibrosarcoma," "osteogenic sarcoma," and so forth.

4. Is it an epithelial growth? If so, it may properly be designated a carcinoma. In this connection, however, it must be observed that neoplasms of various organs of the body constitute such special and peculiar diseases of those organs that specific names should be increasingly employed, such as "orchitoma" for tumors of testis, "nephroma" for those of the kidney, "thymoma" for the thymic growths, and so forth.

5. If the neoplasm is a carcinoma, what is the character of the underlying epithelial element and what is the degree of its differentiation? For example, squamous cell carcinoma of the tongue, grade 2 (on a basis of 1 to 4, inclusive), or adenocarcinoma of the cervix, grade 1+, and so forth.

6. Is the tumor composed of neurogenic elements? If so, it is probably a glioma of some type. Neuropathologists have adopted special designations for these tumors which to a certain extent express their basic element and degree of differentiation: "astrocytoma," "medulloblastoma," and so forth. Several types of neurogenic tumors are not yet clearly enough identified as to their basic element to justify any but special terms, such as "fibroneuroma," "pinealoma," "meningioma," and so forth.

7. Does the neoplasm contain special differentiated tissues? If so, it may be a syncytioma, leiomyosarcoma, rhabdomyosarcoma, teratoma, and so forth.

TERMS THAT SHOULD BE ELIMINATED

In view of the foregoing fundamental assumptions it is suggested that the following designations be eliminated:

1. All so-called "benign" tumors. This is a large order for anyone no matter how iconoclastic he may be. Theoretically it rests on a sound basis, as explained previously. Practically

there are many difficulties, some of them, for the time being, apparently insurmountable. However, that is no reason why a start should not be made.

In almost every treatise on tumors in any language the list begins with "benign" tumors and this list is usually headed by a consideration of the so-called "fibroma." Often it is stated that this is the most common "benign" tumor of any organ or tissue. After more than thirty years I have been unable to find a fitting example of this "tumor" in order to demonstrate its characteristics to students of pathology. So-called "fibromas" of the breast, keloids, nasal polyps, ovarian stroma "fibroids," and pedunculated connective tissue masses on the skin have been repeatedly tested and failed to measure up to any standard of a real neoplasm. Anomalies of growth or development, abnormal involutional and probably functional hyperplasias, or chronic inflammatory fibrosis are usually sufficient to explain the fibrous formation. Finally, a real "oma" composed of fibrous connective tissue must be sarcoma even if its rate of growth permits it to exist for the greater part of a normal lifetime. The term "fibroma" should therefore be deleted from modern tumor nomenclature.

Similarly "chondroma" and "osteoma" belong in the discard. But here one meets with certain real difficulties. Not so long ago I was demonstrating a "chondroma" of the lung. These masses of cartilage in the lungs are still rare enough to deserve comment. One of my students at once placed me "on the spot" by asking if it was a real tumor. I did not know and was forced to admit it. I firmly believe they are maldevelopments of the bronchial cartilage anlage, but some of them have such abnormal proliferative tendencies that I was not surprised when I encountered for the first time an apparently primary chondrosarcoma of the lung. So-called "chondromas" in other locations may offer similar difficulties, and unless an autonomous growth tendency can be demonstrated there may remain doubt as to their proper classification. With "osteomas" the same distinctions are necessary and often they present the same puzzling problem. When they show tendencies to independent proliferation they are, of course, osteosarcomas, otherwise they are not true tumors.

With the "lipomas" my difficulties increase tremendously. Abnormal masses of adipose connective tissue are perhaps the most common of this "benign" group of tumors. They are encountered in practically every organ or tissue of the body; usually small, insignificant, and absolutely harmless, they may occasionally attain huge and dangerous proportions. Moreover, they rarely develop autonomous tendencies and, when they do, liposarcomas are usually readily recognizable. I have no reasonable theory to account for the presence of these abnormal collections of fat. I cannot, however, credit them with neoplastic characteristics.

Concerning "myxomas" the problem is much simpler. These so-called tumors never should have received an independent designation. Whenever interstitial mucus collects in abnormal proportions, a myxedematous appearance develops and this fact constitutes myxedema, whether it is in an organ or tissue in which there clearly is obstruction to the lymphatics, as in elephantiasis, or in hypothyroidism, when a more generalized edema is present, or in a localized mass of smooth muscle or fibrillar connective tissue. "Myxedematous" and not "myxomatous" is the proper descriptive adjective.

There is a peculiar type of slowly growing sarcoma which contains different amounts of adipose connective tissue in various stages of differentiation and fibrillar connective tissue, often with huge amounts of interstitial mucus. These tumors are called "myxoma lipomatodes" or "lipoma myxomatodes." They are really myxedematous liposarcomas.

With the "leiomyoma" often miscalled "fibroid" by even our most cultured surgeons, the question of elimination of the former designation is at least debatable. These localized masses of smooth muscle, encountered so frequently in the uterus, are almost as commonly present in the wall of the stomach of both sexes as well as occasionally in the wall of the esophagus, small intestine, urinary bladder and, more rarely in other locations. Their singularly circumscribed, so-called expansile growth, their

exceedingly slow rate of hyperplasia, and the varying degrees of atrophy of muscle elements with fibrous connective tissue, interstitial mucus, calcareous and even osseous replacement, give them the ideal characters of "benign" tumors and their right to this classification has rarely been denied. But are they really neoplasms in any sense of this word, either benign or malignant? Might they not be considered to be simply expressions of abnormal local hyperplasia of smooth muscle cells produced from isolated bundles by those same physiologic stimuli which produce diffuse hyperplasia in the entire muscular structure of the uterus or other organs containing smooth muscle components? The "fault" may lie in the neurogenic apparatus or some abnormal displacement of the muscle cell groups. The fact that a leiomyosarcoma is rarely found in these same organs or even in these isolated masses is beside the main question. But when I am challenged to suggest a substitute for the term "leiomyoma" without the "oma" signification, I am, without doubt, due for certain defeat. A little ingenuity might hazard "leiomyomilon" meaning mass of smooth muscle, or "topical leiomyon," and so forth, but even a little common sense indicates the effort is hardly worth the candle. Hence I will let leiomyoma rest in peace, even if it is perhaps poorly applied.

Proceeding into the even more complicated field of "benign" epithelial tumors the "adenoma" is at once encountered and it will resist stubbornly any attacks against it. But attacked it must be for it embraces altogether too many diverse conditions and circumstances of glandular epithelial hyperplasia. Many of these, such as adenomas of the liver and pituitary, thyroid, adrenal, and prostate glands, quite manifestly have nothing to do with true neoplastic formations but are present as a response either to reparative tendencies, as in the liver, or to abnormal, and probably hormonal, stimuli as in the pituitary, thyroid, adrenal, or prostate glands. Similarly, hyperplastic islands of Langerhans in the pancreas, certain collections of cells in the ovaries, and other abnormal masses of differentiated cells like the acid cells of the gastric mucosa and Brunner's glands of the duodenum, are probably much better explained on the basis

of unusually applied or abnormal physiologic impulses. Perhaps that is all that any neoplasm is, but the "adenomas" I am considering are in no broad sense autonomous.

Again, however, a substitute nomenclature presents many practical objections. For the "adenomatous" thyroid the German pathologists have long employed the term "struma nodosa," or nodular goiter, and this suggestion is to be strongly commended and the term should be adopted. But "fetal adenoma" still remains to plague us and what about the host of other "adenomas" for which we should find terms more clearly expressing their true significance? One might suggest "nodular" prostate, nodular repair of the liver, and perhaps "topical nodules" of the anterior lobe of the pituitary, adrenal cortex. and of the islands of the pancreas, but again the effort is not particularly worth while. More knowledge of these latter isolated masses of hyperplasia is necessary. Still further objection is raised by the fact that almost by imperceptible stages many of these "adenomas," especially in the pituitary, adrenals and pancreas, become distinctly true neoplasms, that is carcinomas, often with every manifestation of independent growth that any carcinoma may exhibit. Perhaps they are all inherently carcinomas from the start. If so, the term meaning cancer has been made almost ridiculously inclusive.

In this connection, however, must be considered the so-called "adenomas" of the renal cortices. I just shied away from the obvious implications of theoretical considerations concerning new formations in the pituitary, pancreas, adrenals, and perhaps other organs. In the case of the kidney my studies of "adenomas" lead me to the irresistible conclusion that from the very beginning these tiny newgrowths are true neoplasms. Every possible transition, from microscopic collections of cells to frank carcinomas of the kidney, have been observed and the ridiculousness of such an inclusion in the term "carcinoma" is changed to an imperative necessity. Further support for this at first glance too radical conclusion is found in a study of the next group of epithelial "benign" growths, namely, the polyps and papillomas.

For many years I have been studying the cytologic characteristics and neoplastic tendencies exhibited by polyps of the colon. Without pausing too long to marshal all the facts in favor of the concept that all real polyps of the colon are carcinomas, I can only challenge objectors to this view to tell me after a similar study the approximate stage at which these newgrowths are worthy of the name "carcinoma." For by imperceptible stages the tiny, even microscopic polyp, harmless appearing grossly and eminently quiescent clinically, may surely be observed to become a true neoplasm with every character ascribed to this entity.

The same conclusion is reached concerning polyps of the stomach and small intestine, although here, more discretion must be exercised not to confuse projecting ridges of mucosa with polypoid growth. The changed characters of the cells and their disorderly arrangement will usually give the required clues.

I have had very little experience with bronchial polyps, but from biopsy material and a few postmortem specimens I am inclined to regard them all as distinct neoplasms.

In polyps of the uterus, both of the endometrium and of the cervix, for some unknown reason, carcinomatous tendencies can rarely be demonstrated. They apparently belong to a different class of formations. Perhaps, as with polyps of the nasal passages and nasopharynx, inflammatory influences play a major rôle.

The papillomas present a variegated group, several members of which illustrate the thesis I am defending. When I was a young surgical pathologist, one of my major problems was to determine for the urologist whether a given specimen from a papilloma of the urinary bladder was a "malignant" or "benign" growth. If it had not invaded the subepithelial tissues it was pronounced benign. The fact that many of these so-called benign papillomas recurred and often became "clinical" carcinomas led me and many other pathologists and urologists to the conclusion that there is no such thing as a "benign" papilloma of the urinary bladder. They are all carcinomas from the beginning.

For papillomas of the ducts of the breast I am not able to

marshal any such array of facts concerning their neoplastic status. There seems to be a wide difference of opinion among experts in this field. While I am naturally inclined to view them as neoplasms, my only contention is that if it is finally decided that they are not, the "oma" designation should be dropped and a name should be selected which will more closely signify their real character.

Time and my own limitations will not permit me to discuss further the remaining papillomas and polyps, such as those of the walls of the gallbladder, or the larynx, or from skin surfaces and other rarer locations. As in the case of those of the ducts of the breast, they need etiologic clarification and any designation of them as tumors should also imply their neoplastic properties, if this is the fact. Otherwise more appropriate terms should be used.

The hemangiomas and lymphangiomas are not tumors in hardly any sense of the word and these terms should not remain in the tumor classification. Composed, as they are, of dilated lymph or blood vessel channels, often reaching enormous and even dangerous proportions, the true explanation of their formation has not vet been given. The most common site of so-called "cavernous hemangiomas" is in the liver where they are often multiple and where they frequently show regressive changes to the state of complete fibrosis. "Lymphangiomas" most often are recognized in and beneath the capsule of the spleen. Either of these formations may occur in any organ or tissue of the body. Whether they represent an arteriovenous fistula or a congenital maldevelopment of vascular arrangement they certainly should not be regarded as neoplasms. The names used are too old and well established to be easily pushed aside. However, to be consistent I am proposing the terms, "hemangiecton" and "lymphangiecton," as more reasonable substitutes although I shall continue to use the old names.

2. Endothelioma. This term has been employed for pleural neoplasms and has been demonstrated to be incorrectly applied. Similarly it has been used and discarded for so-called meningiomas, mixed tumors (carcinomas) of the parotid and face, tumors

of the testis, and many other tumors whose histologic picture was not too clear. One remaining authentic survival is the so-called "Ewing's" tumor or "endothelioma" of bone. It is to be hoped that this term also may be changed to a more useful and revealing designation. Endothelium is a modified connective tissue and true tumors arising from it must be sarcomas.

- 3. Epithelioma. For a long time this term has been employed by some pathologists and clinicians as a name for carcinomas of squamous epithelial surfaces. Only custom, and a bad one at that, can justify the continued use of the term, for naturally any carcinoma arising, as most of them do, from epithelial cells is an epithelioma. There is really no use at present for this word. The designation "squamous cell carcinoma" is perfectly sufficient to distinguish them from glandular or "adeno" carcinomas.
- 4. Basal cell carcinoma. The theory underlying the use of this name is the supposition that in the epidermis the basal layer of cells, that is, those nearest to the corium, constitute a specialized class of cells and that the slowly growing carcinomas of the skin which contain cells somewhat resembling them in size and appearance, and often closely connected with them, must therefore originate from them. Some histologists express the conviction that this row of cells is the reproductive layer of the "stratum germinativum." And here we have the paradox of a tumor with very sluggish proliferation of cells arising from the germinal layer of an epithelial structure. This fallacious reasoning is wrong in two respects: First, the real "stratum germinativum" of the epidermis is in all probability the malpighian The so-called basal cells are probably only those derived from this layer, which are regimented in a row against the connective tissue, quite evidently for the purpose of facilitating the passage of nutritive lymph to the growing cells. The second point is that the tumors under discussion, in the opinion of some of us at least, are derived from hair follicle epithelium.

It is strange that there has not been a tumor with a name indicating its derivation from the differentiated cells of the epidermis which constitute the nidus for the formation of hair. These highly specialized cells have several layers in a particularly complicated arrangement and they proliferate always in one developmental direction. Moreover they have the inherent capacity to produce melanin more than any other cells of the epidermis. From the hair follicle epithelium originates the pigmented mole and the so-called "basal cell" carcinoma. A much more appropriate term for this tumor would be "trichinoma." It is not by any means impossible that, later, the so-called "malignant melanomas" may also be shown to arise from some portion of this same hair follicle, perhaps from the matrix cells of the medulla.

5. "Colloid carcinoma." The well-known gelatinous appearance of some carcinomas undoubtedly gave rise to this designation for them. A little study, and very little at that, proves that the peculiar gross characteristics of these neoplasms are due to mucus secreted by the epithelial cells which make up the newgrowth. Often this mucus is collected in large quantities and remains in masses after the cells which produced it have disappeared. If any qualifying adjective is needed for such tumors it should be "mucous" (not "mucoid") and certainly not "colloid."

The suggestions which I have just made are directed toward the improvement of the classification of tumors. Many of these suggestions may be proved eventually to be without proper foundations and many of them have certainly been inadequately presented. However, I am firmly convinced the problem is one that deserves serious consideration by everyone concerned with tumor nomenclature. I am equally certain that the subject by no means has been completely exhausted by this brief discussion. In the glioma group, for example, the many transitions which occur between the so-called "spongioblastoma multiforme" and the astrocytoma suggest the possibility that the former term might be eliminated and the astrocytomas be graded according to their degrees of differentiation: astrocytomas, grade 1, grade 2 representing the astroblastoma and grades 3 and 4 the spongioblastomas. Tumors arising in the lymph nodes and bone marrow likewise deserve a more stable nomenclature.

At least such discussions as this may promote controversy and in serious, honest controversy the underlying truths of science usually come to light.

CONCLUSIONS

- 1. The present nomenclature of tumors is in serious need of critical review.
 - 2. The terms "benign" and "malignant" might be dropped.
- 3. The implications carried by the words "benign" tumor are illogical and all "oma" suffixes for these tissue masses should be substituted when possible by more appropriate designations.
- 4. There is no good reason to retain the names "myxoma," "endothelioma," and "epithelioma."
 - 5. The "basal cell" carcinoma is probably incorrectly labelled.
- 6. Further simplification and elimination of unsuitable terms in tumor classification should be attempted.

CHANGES IN SERUM CALCIUM, INORGANIC PHOS-PHATE AND PHOSPHATASE ACTIVITY IN THE PREGNANT WOMAN*

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That the serum calcium tends to diminish during pregnancy, especially in the later months, seems to be decisively established, notwithstanding a number of reports which are in conflict with this observation. The largest series of analyses are those of Mull and Bill¹ who performed nearly 5000 determinations on a group of 900 subjects. Mull's results show an average fall of approximately 5 per cent. The decline is progressive as pregnancy advances, but is interrupted six to seven weeks before delivery; then there is a slight rise until delivery, followed by a sharper elevation after delivery.

In a group of non-pregnant women studied by Oberst and Plass² the average serum calcium was found to be 10.4 mgm. per 100 cc., which compares with the average of 10.6 mgm. reported by Mull and Bill,³ who also found that 94 per cent of their values fell between 10.0 and 11.5 mgm. Oberst and Plass² saw no change early in pregnancy (variations between 9.8 and 10.9 mgm; average 10.4 mgm.), but during the eighth and ninth months the concentrations varied between 8.8 and 10.8 mgm., the average being 9.5 mgm. During labor the average was restored to

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9.9 mgm. (range of 8.9-11 mgm.) and remained at this level during the succeeding 7-9 days of observation.

An earlier study by Plass and Bogert⁴ disclosed that of 105 subjects examined in the last five months of pregnancy 50 per cent had serum calcium values below 9 mgm. per 100 cc., this being the lowest value which they observed in normal nonpregnant women. Such a high incidence of hypocalcemia does not seem to have been encountered by other investigators. However low serum calciums occur with sufficient frequency to justify further study of the hypocalcemic tendency.

It would be of fundamental importance to determine whether hypocalcemia is a normal condition of pregnancy and the extent to which the serum calcium level during pregnancy is influenced by racial, climatic, seasonal, nutritional, hormonal and other

factors.

The analyses to be reviewed in this paper were undertaken principally with the thought that if a sufficiently large group of pregnant women were studied some might be found in whom the occurrence of hypocalcemia could be definitely related to parathyroid deficiency. Experimental studies in this laboratory have led us to conclude that pregnancy is normally associated with stimulation of parathyroid activity, and consequently an inadequate response would represent a condition of relative hypoparathyroidism and should result in hypocalcemia.

Aside from this consideration it seemed desirable to obtain data for a Southern latitude, since practically all other studies have been conducted in Northern cities (Cleveland, Detroit, 4 Iowa City,² Philadelphia,⁵ etc.). That climate is a factor in calcium metabolism is illustrated by the observations of Coons⁶ who found that pregnant women in Chicago (lat. 41.9) require more calcium than pregnant women in Stillwater, Oklahoma (lat. 36.1). The difference in efficiency of calcium utilization in the two groups was attributed by Coons to the difference in the amount of sunshine in the two localities.

The present study included determinations of inorganic phosphate and phosphatase. Serum protein was determined only for the purpose of testing the conclusion of Oberst and Plass² that the fall in serum calcium during pregnancy is unrelated to changes in serum protein. Our results in this regard confirm those of Oberst and Plass.

Serum calcium was determined by the Clark-Collip modification of the Kramer-Tisdall method. Inorganic phosphate and phosphatase were determined according to the methods of A. Bodansky.⁷

The subjects were patients in the antenatal clinic of the John Sealy Hospital. Many of these attended with sufficient regularity to enable us to collect from six to twelve blood specimens at intervals of two to four weeks. Fewer specimens were obtained from those who attended the clinic with less regularity. The present report is based on the data obtained in a total of 300 subjects.*

INDIVIDUAL RESULTS, TYPICAL AND ATYPICAL EXAMPLES

The changes in calcium, phosphorus and phosphatase are not alike in all pregnant women. Individual differences occur, but in the greater number the changes are fairly uniform.

A more or less typical example is illustrated in figure 1 (subject A). At 112 days antepartum, the serum calcium in this case was 10.3 mgm. During the succeeding four weeks it declined to 9.4 mgm., at which level it remained until the 56th day antepartum. A further reduction to 9.2 mgm. occurred during the tenth lunar month. The serum calcium during labor was 9.6 mgm.

The concentration of inorganic phosphate tends to increase during pregnancy, and especially at the time of labor, but as a rule the change is small and the normal concentration is not exceeded. On the other hand, a significant rise in serum phosphatase activity occurs almost invariably. In subject A it increased from 3.2 units† at 112 days to 8.7 units at term.

^{*} Acknowledgement is due to Dr. Willard R. Cooke, professor of obstetrics and gynecology, and to the members of his staff for their coöperation in making these patients available for our study.

[†] Phosphatase activity throughout this paper is expressed in terms of A. Bodansky units.

The observations in subject B were less characteristic. Except for the temporary drop to 10.1 mgm. at 77 days antepartum the serum calcium remained at 10.5 mgm. until two weeks before parturition. The inorganic phosphorus rose from 2.9 mgm. to about 3.67 mgm. There was relatively little increase in serum phosphatase (3.7 units at 98 days; 4.8 units at term).

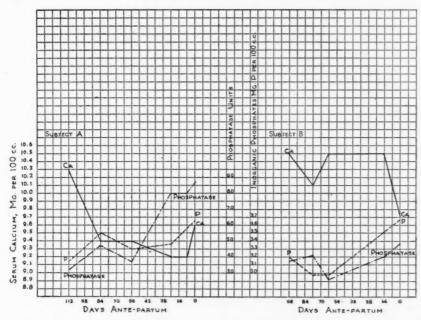


Fig. 1. Showing characteristic changes in serum calcium, inorganic phosphorus and phosphatase in subject A, and somewhat less typical changes in subject B.

A somewhat different and definitely atypical course of events is illustrated in figure 2. The serum calcium in subject O. H. fell to 8.9 mgm. at 63 days antepartum and reached a minimum of 8.6 mgm. at term. The inorganic phosphate increased to a maximum of 5.6 mgm. The most conspicuous changes were observed in respect to the phosphatase. Even at 77 days antepartum this was abnormally elevated (7 units). Three weeks before delivery it was 13 units and reached a maximum of 32 units at term. This is the highest value for phosphatase activity which we have observed in our series of pregnant women. The subject, a 19 year-old negro primipara was apparently well nourished and gave birth to a normal baby. Jaundice in the mother was definitely excluded. Five weeks after delivery, blood

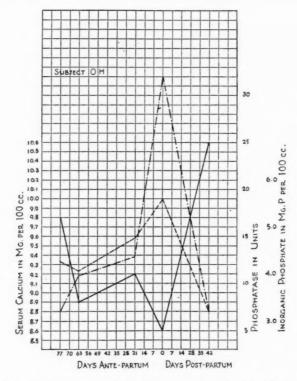


Fig. 2. Showing an abnormal rise in serum phosphatase (dots and dashes) toward the end of pregnancy in subject O. H. Note also the low serum calcium (solid line) at term and restoration to approximately normal after parturition.

was obtained for analysis. The serum calcium and phosphorus were restored to normal, (10.6 and 3.2 mgm., respectively). The phosphatase activity was equivalent to 7 units, a value which is definitely above the non-pregnancy, non-lactation normal (1.5–4 units in adults).

Calcium

The trend which is typical for the individual characterized the entire group. From an average of 9.8 mgm. during the third to the sixth lunar months the serum calcium declined to an average

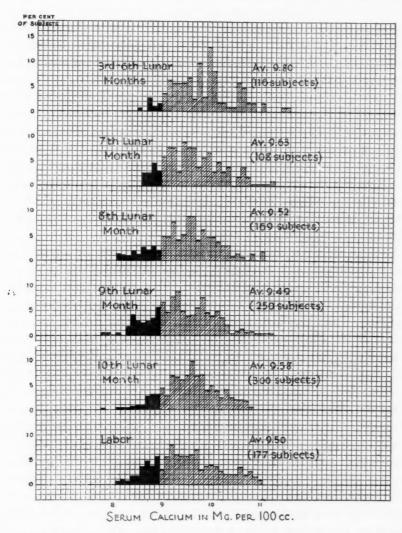


Fig. 3. Frequency of distribution of the serum calcium concentrations at various stages during pregnancy.

of 9.63 mgm. in the seventh, 9.52 mgm. in the eighth and 9.49 mgm. in the ninth. The decline was slightly reversed in the tenth month, the average being 9.58 mgm. During labor the average serum calcium for 177 subjects was 9.50 mgm.

In figure 3 are represented the range of values and frequency of distribution of the serum calcium concentrations for each of these periods. Attention is directed particularly to the increasing frequency of values below 9 mgm. as pregnancy advanced. During the third to the sixth lunar months only six per cent of the concentrations fell below 9 mgm. In the seventh month 13 per cent were below 9 mgm. and in the eighth month the low values increased to 15 per cent of the total. A sharper increase occurred during the ninth month, 24 per cent of the concentrations falling below 9 mgm. In the tenth lunar month the trend in the direction of subnormal concentrations was reversed even more sharply than the average. Only 13 per cent of the serum calciums were below 9 mgm. However, this was not sustained for at the time of labor the incidence of serum calciums below 9 mgm. had again increased to 23 per cent.

Such a high frequency of hypocalcemia must be significant. An explanation may be sought in the nutritional status of the patients, but it is also conceivable that the results reflect relative parathyroid deficiency in a considerable proportion of pregnant That the nutritional factor is important seems probable. It is only necessary to examine Maxwell's data⁸ on pregnant Chinese women to realize the profound effect of diet on blood calcium. In some of Maxwell's subjects the serum calcium fell below 4 mgm. per 100 cc. However, it is to be observed that the calcium intake of many of these women averaged only about 0.1 gram per day and, moreover, the calcium reserves of many of them were in a depleted state at the onset of pregnancy. While it is not improbable that even moderate degrees of hypocalcemia may be caused by dietary calcium deficiency, the general experience has been that within comparatively wide limits the level of calcium in the food has little effect on the serum calcium concentration (Pyle, Potgieter and Comstock⁹). It may also be noted that an adequate or even a superabundant calcium intake does not necessarily assure the maintenance of the serum calcium at the normal non-pregnancy level. A positive calcium balance during pregnancy is entirely consistent with moderate hypocalcemia.

The function of the parathyroids in maintaining the serum calcium above the tetany level is even more decisive in the gravid than in the non-gravid organism. Even on an adequate calcium intake the parathyroids increase in size and apparently in functional activity. It is therefore logical to assume that inadequate stimulation of the parathyroids would result in relative hypoparathyroidism and hypocalcemia. The degree of stimulation necessary to maintain the serum calcium within the normal pregnancy range is probably influenced by nutritional factors. Experiments in this laboratory indicate that during pregnancy there is an approximate reciprocal relationship between the level of calcium intake and the degree of parathyroid enlargement.

The maintenance of a certain level of serum calcium under conditions of calcium deprivation would obviously call for a higher level of parathyroid activity than under conditions of abundant calcium (and vitamin D) intake. Failure of the parathyroids to respond adequately to the demands of a given situation would result in relative hypoparathyroidism. However, even though we have observed many instances of hypocalcemia in our series, it has been impossible to attribute them specifically to parathyroid deficiency. Nor were we able to explain all of the low values on the basis of nutritional deficiency. although in those instances where the serum calcium fell below 8.5 mgm. this seemed to be the predominant factor.

Seasonal influences have been demonstrated by Mull and Bill.¹ For all periods of gestation they obtained lower serum calcium values during the months January to May than during June to December. We have encountered similar seasonal differences. with the averages at higher levels during August, September and October than during January, February and March. The difference between the extremes was nearly 0.5 mgm. These findings are perhaps related to the amount of sunshine. During January, February and March the total hours of sunshine were

70, 119 and 138, respectively, while during each of the months, August, September and October, the total hours were well in excess of 200. Although the relationship between sunshine and blood calcium seems reasonable there is however the possibility that the seasonal effect may not be determined altogether by the hours of sunshine. There is some evidence to suggest rhythmic seasonal fluctuation in serum calcium. This is relatively inconspicuous in the normal non-pregnant adult under ordinary conditions of calcium metabolism, but under the altered conditions of bone metabolism such as characterize pregnancy and rapid growth in children, the seasonal influence becomes more apparent.

Another question concerns the tendency to hypocalcemia despite the apparent increase in parathyroid activity. Is it possible that the serum calcium is depressed below the non-pregnancy level through the agency of some factor which antagonizes the action of the parathyroids? It is perhaps a matter of no small significance that the serum calcium rises quite sharply after parturition which suggests the abolition of the calcium-depressing effect. In this connection it may be recalled that many years ago Frommer¹⁰ induced active tetany in parathyroid-deficient dogs by the administration of placental extract. These considerations indicate that the placenta itself may be involved in serum calcium regulation, but before this view can be adopted it will be necessary to demonstrate the occurrence in the maternal portion of the placenta of a substance capable of lowering the serum calcium.

If the existence of such a substance were discovered, its participation in the regulation of the blood calcium would be of unusual importance from the standpoint of calcium conservation. From the work of Pyle and associates it would seem that there is a close correlation between serum calcium and fecal calcium. Presumably a moderate degree of hypocalcemia affords an advantage to the gravid organism because it is accompanied by a diminished transfer of calcium from the blood to the bowel. Because of this and other considerations we propose the view that the reduction in serum calcium during pregnancy does not

necessarily represent an abnormal condition and that there are in all probability two distinct normal levels, one for nonpregnant women and a somewhat lower level for pregnant women.

Inorganic phosphate

Mull and Bill¹ observed a gradual decline in serum phosphorus from 3.43 mgm. per 100 cc. at 29 to 32 weeks before delivery to 3.24 mgm. at 11 to 13 weeks before delivery. During the remainder of pregnancy a gradual recovery occurred, followed by an abrupt rise after delivery. In our analyses the averages changed comparatively little. From figure 4, in which are charted the frequencies of distribution, it will be noted that for

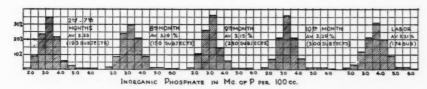


Fig. 4. Frequency of distribution of serum inorganic phosphorus at various stages during pregnancy.

the second to the seventh lunar months the average was 3.23 mgm. The average was 3.19 mgm. during the eighth month, 3.15 mgm. during the ninth month, 3.29 mgm. during the tenth month and 3.21 mgm. at the time of labor. Practically all the data were within the range of 1.5 to 6.5 mgm. per 100 cc.

Phosphatase

In marked contrast to the relative constancy of the inorganic phosphate throughout pregnancy were the changes in phosphatase activity. Up to the seventh lunar month the majority of results, namely 83 per cent, were within the normal range for adults, i.e., 1.5 to 4 units. Fifteen per cent were between 5 to 6 units. A conspicuous change in the pattern of distribution became evident during the seventh month when less than 40 per cent of the values fell below 3 units as compared with over 60 per cent during the

earlier months. Forty-six per cent of the data were between 3 and 4 units.

The shift to the right progressed in the succeeding months. During the eighth lunar month only 30 per cent of the phosphatase values were below 3 units. However, even at this time, nearly 60 per cent were within the normal nonpregnancy range of variation.

The ninth month was marked by a further decline in the number of low figures and a corresponding increase in the number which exceeded 4 units. Altogether only 40 per cent of the values were below 4 units, while approximately 20 per cent of the results were above 6 units. In the tenth month only 18 per

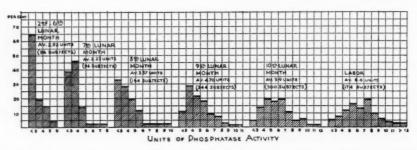


Fig. 5. Frequency of distribution of serum phosphatase activity at different stages during pregnancy.

cent of the results were within the usual normal range. At the time of labor 87 per cent of the data were above 4 units. We see therefore that a reversal in distribution took place. Even as late as the seventh lunar month over 80 per cent of the data were within the normal range of variation, while in the last month of gestation well over 80 per cent were above the normal limits.

Turning to average values it will be observed that for the second to the sixth lunar months, the mean was 2.92 units. The average rose progressively to 3.23 units in the seventh month, 3.57 in the eighth, 4.7 in the ninth, 5.9 in the tenth month, and 6.6 units at the time of labor.

Since this work was begun Meranze, Meranze and Rothman¹¹ have reported a rise in serum phosphatase activity during preg-

nancy and have offered various interpretations for it. One is based on the coincidence of the rise in blood phosphatase with the period of greatest activity of fetal ossification and presumably therefore with the period of greatest phosphatase need. It is pointed out that the fetus itself does not elaborate much phosphatase at this time and that the rise in maternal phosphatase may represent a successful compensatory response in the mother.

Meranze and associates also raise the question as to whether the elevated blood phosphatase may not represent a pathologic or potentially pathologic state of bone metabolism in mother or fetus.

We are inclined to relate the progressive rise in phosphatase during pregnancy to the increased lability of the bone metabolism of the maternal organism. A. Bodansky and Jaffé¹² have put forward the view that the serum phosphatase is an expression of the "controlled specific reactivity" to resorption of bone (normal and pathologic). To illustrate, in normal children the specific reactivity to the resorption of bone is at a higher level than in adults. This accounts for the higher level of phosphatase, but since even at the higher level the specific reactivity is controlled, the phosphatase is maintained within certain relatively narrow limits (5-14 units). The lower values in normal adults (1.5-4 units) reflect a lower specific reactivity (to slower resorption) and a slower normal formation of bones.

According to A. Bodansky and Jaffé, 12 specific reactivity is stimulated in osteoporosis in accordance with the degree of resorption; "however, as is obvious, synthesis of bone is inadequate because of the lack of an essential substance or mechanism; adequate controls are therefore not set up; and serum phosphatase increases—most in the young, moderately in the middleaged and slightly or not at all in the senile."

The development of varying degrees of demineralization in pregnancy, especially under conditions of inadequate mineral intake, is most probable. Indeed some depletion of the calcium and phosphorus reserves of the bones may occur even when the intake of these elements is fairly satisfactory. In experimental studies on rats in this laboratory it has been possible to show by X-ray some degree of demineralization of the long bones even when the intake of calcium and phosphorus was optimal for reproductive success (Diet 7 of Cox and Imboden¹³). As might be expected, demineralization was much more severe on a deficient intake of calcium (Diet 26 of Cox and Imboden¹³). Such bone rarefaction was not apparent however in parathyroidectomized pregnant rats, from which it may be concluded that the parathyroids were probably involved in the process of demineralization which occurred in the normal animals.

It may be assumed that the parathyroids control the removal of calcium from the bone when this is necessary for the maintenance of the serum calcium above the tetany level. During pregnancy, owing to the demands for fetal growth, the tendency to hypocalcemia increases, but this is ordinarily compensated for by an increase in parathyroid activity.

We may therefore look upon pregnancy as a condition ordinarily associated with some degree of hyperparathyroidism. A. Bodansky and Jaffé¹² have indicated that in hyperparathyroidism the generalized resorption of bone stimulates specific reactivity. Resorption in hyperparathyroidism is more active than in generalized osteoporosis; hence specific reactivity and serum phosphatase are controlled at a high level.

Our data on phosphatase in pregnant women suggest that in a large proportion of cases the level of specific reactivity to bone resorption is moderately elevated and that it is controlled at this higher level. However, the occasional high value, such as the 32 units observed in subject O. H., probably reflects an abnormally high level of specific reactivity, which is presumably related to more than the usual amount of parathyroid stimulation seen in pregnancy.

On the other hand, the maintenance of low serum phosphatase during pregnancy may also be significant. In our experimental studies we have observed that the serum phosphatase of parathyroidectomized rats at term is distinctly lower than the serum phosphatase of normal rats at term. The other important difference is in respect to the serum calcium which is usually

depressed below 5 mgm. in parathyroid-deficient rats, but which remains in the neighborhood of 10 mgm. in unoperated normal pregnant animals.

These and other considerations suggest that in human subjects the combination of low phosphatase-low calcium toward the end of pregnancy may be related to a depressed level of specific reactivity of bone which is in turn referable to relative parathyroid insufficiency.

SUMMARY

This report is based on determinations of calcium, phosphorus and phosphatase in a group of 300 pregnant women.

From an average of 9.8 mgm. during the second trimester, the serum calcium declined to an average of 9.63 mgm. in the seventh month, 9.52 mgm. in the eighth, and 9.49 mgm. in the ninth. rise to 9.58 mgm. occurred during the tenth lunar month, but at term the average for 177 subjects was again reduced to 9.50 mgm.

Attention is directed to the increased frequency of hypocalcemia (values below 9 mgm.) as pregnancy advances. Only 6 per cent of the determinations were below 9 mgm. during the second trimester. In the seventh month 13 per cent were below 9 mgm.; in the eighth, 15 per cent; in the ninth 24 per cent; in the tenth month the trend was reversed, only 13 per cent being below 9 mgm.; however, by the time of labor the incidence of low values had again increased to 23 per cent.

For the later stages of gestation (eighth to tenth lunar months) the averages for serum calcium were somewhat lower during the months of January, February and March than during August, September and October.

The significance of pregnancy hypocalcemia is briefly discussed from the standpoint of nutrition, parathyroid function and seasonal influences. The possibility that the placenta participates in the regulation of the serum calcium concentration in the maternal circulation is indicated. It is also suggested that a moderate reduction in serum calcium during pregnancy does not necessarily represent an abnormal condition.

The frequencies of distribution of inorganic phosphate were

determined for the various stages of pregnancy. For the second to the seventh lunar months the average was 3.23 mgm.; for the eighth month it was 3.19 mgm.; for the ninth month, 3.15 mgm.; for the tenth month it was 3.29 mgm.; during delivery the average was 3.21 mgm.

The frequencies of distribution of phosphatase were also determined. Up to the seventh lunar month, the majority of results, namely 83 per cent, were within the normal range for adults, i.e., 1.5 to 4 units. A marked shift to higher phosphatase values was apparent in the seventh month and became more and more conspicuous as pregnancy advanced. In the last month of gestation well over 80 per cent of the data were above the normal limits.

For the second to the sixth lunar months the average phosphatase activity was 2.92 units. It rose progressively to 3.23 units in the seventh month, 3.57 units in the eighth, 4.7 units in the ninth, 5.9 in the tenth month and 6.6 units during delivery.

The significance of the rise in phosphatase during pregnancy is briefly discussed and its relation to increased parathyroid function is suggested.

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THE PROTECTIVE ACTION OF ALCOHOL IN EXPERIMENTAL TRICHINOSIS*

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Since there are reports that the liberal use of alcoholic beverages while ingesting trichinous meat offers protection against infection and since the use of generous quantities of alcoholics has been advised as therapy in trichinosis, we have investigated this problem by *in vitro* studies and by experimentation on rats.

Our *in vitro* studies showed that a concentration of 25 per cent ethyl alcohol had little direct action on free trichinella larvae but that concentrations as low as 9 per cent interfered with digestive excystment of larvae.

The following studies were made to observe the effects of ethyl alcohol on experimental trichinosis in rats.

METHODS

Twelve adult female white rats of the Wistar strain averaging about 250 grams in weight were segregated into four groups of three each. After a 24 hour fasting period, all were infected with known quantities of *Trichinella spiralis* and all were kept on the same Addis standard dry rat diet but half received ethyl alcohol by mouth while the others served as controls. Eosinophil counts and weights were recorded weekly. Blood alcohol determinations were made at the close of the experiments by Dr. Henry Newman.³ Six weeks after infection all animals were sacrificed, skinned, eviscerated, minced, and digested by artificial gastric juice^{4, 5} and the number of larvae counted for each rat.

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Experiment 1. Effect of alcohol on rats infected with free trichinella larvae

By means of a small stomach tube, six rats under light ether anesthesia were each given 2380 living trichinella digested free of their capsules. Three of these rats also received 4 ml. of 25 per cent ethyl alcohol (group 1). The other three served as controls (group 2). The alcoholized rats remained in a comatose state for almost three hours while the controls rapidly assumed their normal activity. Thereafter, the two groups, in separate cages, were handled exactly alike except that group 1 drank 10 per cent alcohol and group 2 drank water.

TABLE 1
EFFECTS OF ALCOHOL ON EXPERIMENTAL TRICHINOSIS IN RATS

EXPERI- MENT NUMBER	GROUP NUMBER	WEIGHT		HIGHEST	ABSOLUTE	BLOOD	NUMBER OF LARVAE		
		Initial	Final	EOSIN.	CONSUMED	ALCOHOL	Given	Recovered	
		grams	grams	per cent	ml,	mgm. per cent			
1 {	1	750	760	6-9	243	1-8	7140	12,400	
	2	760	755	7-14		0-1	7140	24,370	
11 {	3	755	735	9-16	225	1-69	7650	107,960	
	4	805	695	9-15		0-0	7650	1,008,250	
(5	765	590				6750	323,770	
III	6	835	705		190		6750	314,480	
	7	725	720		3		6750	70,500	

- Group 1. Free trichinella larvae plus alcohol for 6 weeks.
- Group 2. Free trichinella larvae (controls).
- Group 3. Trichinous meat plus alcohol for 6 weeks.
- Group 4. Trichinous meat (controls).
- Group 5. Trichinous meat (controls).
- Group 6. Trichinous meat followed in 48 hours by alcohol for 7 weeks.
- Group 7. Trichinous meat simultaneously with a single dose of alcohol.

Table 1 gives a summary of the observations on this experiment. The initial eosinophil counts of 2–5 per cent rose to 6–14 per cent during the third week of infection. Although there was a total consumption of 243 ml. of absolute alcohol by Group 1, the final blood alcohol figures were not significantly above the normal values of 0–5.0 mgm. per cent. The rats were sacrificed six weeks after infection. The total number of larvae yielded by group 1 was 12,400. The ratio of the total number of larvae given to the total number recovered was as 1 is to 1.7. Group 2 yielded 24,370 larvae, giving a ratio of 1 to 3.4, or twice the yield of group 1. None of the six rats showed noticeable ill effects of the experiment. It is apparent that although alcohol diminished the yield of larvae by half it did not protect against infection.

Experiment 2. Effect of alcohol on rats infected with trichinous meat

This experiment is similar to experiment 1, but the six rats were infected by trichinous meat instead of free larvae. One gram of minced rat meat containing 2550 trichinella larvae was fed each rat. Three infected rats (group 3) were immediately given 4 ml. of 25 per cent ethyl alcohol while the remaining three (group 4) served as controls. Thereafter group 3 drank 10 per cent alcohol and group 4 drank water.

Table 1 summarizes the results of this experiment. Group 3 consumed a total of 225 ml. of absolute alcohol and the final blood alcohols ranged from 1.2 to 69.0 mgm. per cent. Possibly the rat giving the high figure drank a goodly quantity of 10 per cent alcohol just prior to being bled. Both groups lost weight but Group 4 was striking in its loss of over 100 grams or 13.5 per cent of its original weight. The rats in this group were thin and sluggish with ill kempt, sparce fur and showed evidences of having bitten their limbs. The eosinophils mounted to a maximum height of 9–16 per cent in the third week of the infection. The total yield of larvae in Group 3 was 107,960 or a ratio of intake to output of 1 to 14. Group 4 yielded a total of 1,008,250, an intake-output ratio of 1 to 131, or 10 times the yield in Group 3. In this experiment alcohol caused a 90 per cent reduction in the number of trichinella encysting in the muscles of rats infected by trichinous meat.

It was obvious from these experiments that alcohol in large quantities materially reduced the severity of infection from *Trichinella spiralis*. The question which naturally arose was the time at which the ingestion of alcohol is of value in trichinosis. The following experiment was devised to clarify this question.

Experiment 3. Localization of the time of beneficial effects of alcohol following the ingestion of trichinous meat

Nine adult female white rats averaging about 250 grams in weight were segregated into three groups of three rats each. After a 24 hour fast, each rat was offered a quantity of rat meat containing 2250 encysted trichinella, which was rapidly and completely consumed. Thereafter all rats were kept on the same basic diet. The rats of group 5 had water available at all times and served as controls. Those of Group 6 had water available for the first 48 hours but thereafter drank only 10 per cent ethyl alcohol. The rats of group 7 were given 4 ml. of 25 per cent ethyl alcohol immediately following the ingestion of the infected meat. After recovery from the alcoholic intoxication these rats drank water for the remainder of the experiment. Experiment number three ran for almost seven weeks. At the end of this period the number of larvae was counted in each rat.

Table 1 summarizes the observations on this experiment. Group 5, the controls, yielded a total of 323,770 larvae. This gives a ratio of the number of larvae ingested by the group to the number recovered of 1 to 48. In terms of "dressed" rat weight there were 845 larvae per gram. Group 6, which drank 10 per cent alcohol throughout the experiment except for the first 48 hours, gave similar results with a total yield of 314,480 larvae or an intake-output

ratio of 1 to 47. Group 7, which received only an initial inebriating dose of alcohol and thereafter was handled as were the controls, showed a striking diminution in the total yield of larvae. There were only 70,500 recovered from the three rats or an intake-output ratio of 1 to 10 and a "dressed" weight yield of 170 larvae per gram. In this experiment a single dose of alcohol at the time of ingesting trichinous meat reduced the number of trichinella encysting in the muscles of rats by 80 per cent. Practically no protection was afforded by continuous use of large amounts of alcohol over a seven weeks period begun 48 hours after infection.

Data was kept on the consumption of the standard dry rat diet by each group throughout the experiment. Although group 6 did not consume so large an amount of the diet as did groups 5 and 7, the caloric intake was kept practically the same by the additional use of alcohol.

The loss of weight during the experiment was practically identical in groups 5 and 6, being 22.9 per cent of the total original weight in group 5 and 22.5 per cent in group 6. These rats like those of group 4 were sluggish, with sparce fur and superficial skin infections and showed lacerated extremities, undoubtedly resulting from bites made in efforts to alleviate the pains associated with migration and encystment of the trichinella larvae. The rats in group 7 maintained their original weight and showed no ill effects of the experiments.

DISCUSSION

The consumption of alcohol by the rats in these experiments is worthy of comment. Assuming that each rat in groups 1, 3, and 6, having access to alcohol at all times, drank about the same amount, the average daily consumption of absolute alcohol was 1.66 ml. for each 250 gram rat or 5.64 ml. per kilogram. The rats in group 1 consumed as high as 7.52 ml. per kilogram per day. In terms of a 70 kilogram man this would appear to amount to a daily consumption of some 400 ml. of absolute alcohol but in view of the work of Newman and Lehman⁶ this figure may more correctly be placed at about 200 ml. These workers found that the rate of fall of blood alcohol in man is approximately .0025 mgm. per ml. per minute, and in normal rats approximately .0044 and in alcohol habituated rats .0051, thus indicating that the rat burns alcohol approximately twice as fast as man and presumably could metabolize twice the quantity per day. A rat receiving 4 ml. of alcohol per kilogram as a single dose becomes as inebriated as a man receiving the same dose but the rat recovers twice as fast since he burns the alcohol at twice the rate of man. Even a spiritus intake equivalent to 200 ml. of absolute

alcohol is far above that customarily prescribed medicinally, and, according to the rat experiments, would be of no material value after the trichinella had been excysted. Considerably less than this amount of alcohol taken in a single dose while consuming or shortly after consuming trichinella infested meat should in a large measure reduce the severity of but not completely prevent infection.

Our *in vitro* experiments showed that concentrations of alcohol as low as 9 per cent materially interfered with the digestive liberation of encysted trichinella. Our experiments on rats indicate that the same mechanism is responsible for the protective action of a single dose of alcohol taken simultaneously with infected meat. These findings are in accord with those of Blotner, who demonstrated that alcohol destroyed the proteolytic activity of certain gastrointestinal enzymes *in vitro* and *in vivo*. Kreuger and MacIntosh, and Babkin, have shown that alcohol evokes a flow of gastric juice of high acidity but low digestive power.

The number of trichinella larvae recovered from the muscles of the rats of groups 1 and 2, receiving the free larvae, was surprisingly small. This probably indicates that the digestive procedures necessary to the freeing of the larvae prior to their being fed to the rats is not without harm to the parasites. The intake-ouput ratio for the other groups varied from 1–47 to 1–131 with the exception of group 7 where the initial dose of alcohol reduced the ratio to 1–10.

We have expressed the opinion that the quantity of parasites invading the human body is probably the greatest factor in determining the clinical course of the illness.⁵ These experiments on rats strengthen our belief. Certainly the rats of groups 4, 5, and 6 with the heaviest infestation were ill of their trichinosis, one having died in group 5, while those of the other groups apparently were comparatively normal even though, as in group 7, there were 170 larvae per gram of "dressed" rat.

CONCLUSIONS

1. We conclude that while alcohol has little direct action on trichinella larvae in vitro and only halves the number of larvae

developing in the rat after ingesting simultaneously a fixed dose of free trichinella, it may reduce the severity of the infection by 80 per cent if taken at the same time as trichinous meat.

- 2. A single dose of alcohol given simultaneously with trichinous meat reduced the number of trichinella encysting in the muscles of rats by 80 per cent, but when taken in large quantities over a period of seven weeks during the maturation, larval bearing, migrating and encysting stages of the parasites there was no protection.
- 3. In vitro experiments suggested that this protective action was due to alcoholic interference with the digestive liberation of encysted trichinella. Experiments on rats verify these conclusions.
- 4. Daily ingestion of 6–8 ml. of absolute alcohol per kilogram of rat did not appreciably raise their blood alcohol values. Rats burn alcohol approximately twice as fast as man.
- 5. Rats infected with trichinella showed the highest blood eosinophilia during the third week of infection.

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NUTRITIONAL ANEMIA, CLINICAL AND EXPERIMENTAL STUDIES*

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Although the literature is voluminous on the therapeutic value and use of iron in the treatment of secondary anemias, the best form of administration is still controversial. Various phases of the subject have been emphasized at different periods. At one period, emphasis was placed on the differences between organic and inorganic forms, at another upon differences between the ferrous and ferric salts and also the soluble versus the insoluble iron salts. In recent years the greater part of the experimental work done along this line has been upon the effect of combining iron with some other metal, especially copper. The literature, which has been thoroughly reviewed by Hall,3 Meyer4 and Robscheit-Robbins,⁵ will not be discussed here. Experimental work with rats has been done by Mitchell and co-workers,7,10,11 Waddell, Elvehjem, Steenbach and Hart, 12 and Beard and his co-workers in a series of papers.6 The average practicing physician is not interested in the long experimental controversy concerning the various complicated salts and their effects, but would like to know what the best available therapeutic measures are for the treatment of his case. We have undertaken to show the comparative values of some of the most common forms of iron used in the treatment of secondary anemia. The following forms of iron, which are available at all drug stores and in common use to-day were chosen: (1) Arsenoferratose (Sol. Sodium

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Arsenoferrialbuminate);* Arsenoferratose with Copper*; (3) iron and ammonium citrate; (4) Ferratose* and (5) iron pyrophosphate.

Rats from our own stock were used in the experiment. These were weaned at approximately 3 weeks of age. Groups of seven rats each were then placed in iron cages provided with screen bottoms, according to the method used by Elvehjem and Kemmer¹ for producing nutritional anemia in rats. At this time, the hemoglobin averaged 8.6 grams per 100 cc. of blood, and the red blood cell count 5,350,000 per cu. mm. of blood. The Haden-Hauser method of hemoglobin determination was used throughout the work with U.S. standardized pipettes. Red blood counts were done with the improved Neubauer counting chamber and U.S. standardized pipettes. A series of 62 normal rats were used to determine the normal blood cell count and hemoglobin estimation. On the day of birth, the average hemoglobin was 9.4 grams per 100 cc. This rapidly fell each day until on the eighteenth day the lowest point of 5.5 grams was reached. By the twenty-third day of life, the hemoglobin was again up to 8.1 grams. By the twenty-seventh day the hemoglobin was well over 10 grams at which point it leveled out. These were normal breeding groups of rats. The mother was fed in the cage with the young ones, on the usual stock diet.

The red blood cell count on the day of birth was 3,300,000. On the sixth day this began to drop and on the eleventh day reached the lowest point of 3,050,000. Then began a rise, gradual for the first few days, and then rapid, until on the twenty-fourth day the red cells averaged 5,200,000. On the twenty-ninth day, the count had reached 6,100,000 at which point it more or less leveled out (see chart 1).

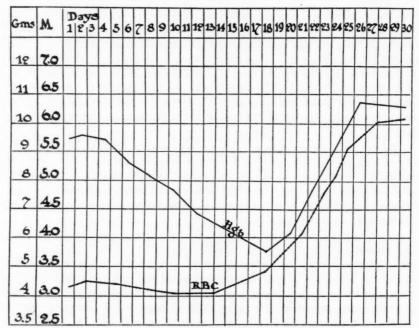
At approximately three weeks of age, the rats were weaned and placed on a diet consisting entirely of dried Klim milk, as used by Harris.² (A large number of adult rats were put on the same milk diet for a period of four months, with little or no effect upon the hemoglobin or red blood cell count.) The rats gained weight and appeared normal. At thirteen weeks of age (average for all groups) the hemoglobin had fallen to an average of 4.17 grams for 100 cc. of blood. If the rats were allowed to continue on this diet, the hemoglobin continued to fall until it reached about 3 grams or below per 100 cc., and the animal soon died.

When the hemoglobin reached an average of 3.7 grams per 100 cc. of blood, three groups of animals were given 2 min. of Arsenoferratose per rat per day along with the regular milk diet. At the end of two weeks, the hemoglobin was up to 7.25 grams per 100 cc.; at the end of four weeks of treatment, the hemoglobin increased to 9.5 grams; and at the end of eight weeks the hemoglobin

^{*} These preparations were supplied by Rare Chemicals, Inc., Nepera Park, N. Y.

globin had reached 12.0 grams and then leveled out at this point. The red blood cell count showed a corresponding increase, rising from 4,400,000 to 7,580,000 per cu.mm. in the eight weeks period (chart 2). This group was receiving daily 0.47 mgm. of free-available iron. A similar group was placed on a smaller dose, receiving daily 0.18 mgm. of available iron. The eventual blood picture was the same, but recovery was slower. At the end of two weeks, the hemoglobin was 5 grams per 100 cc., while the red blood count was 5,575,000. At the end of eight weeks, the hemoglobin was 7 grams per 100 cc. and at 14 weeks the hemoglobin had reached 12.6 grams per 100 cc. of blood.

Another group was placed on 2 min. per rat per day of the Arsenoferratose



Normal rats studied daily until one month of age

vehicle alone. The hemoglobin in this group continued to drop, similar to the control group, and the animals all died, around the fourteenth week of treatment.

Another group was put on 2 min. of Ferratose per rat per day. This group showed even a more spectacular rise than the group on Arsenoferratose. The average hemoglobin rose from 4.6 to 14 grams within seven weeks after treatment was started. The red blood count increased from 4,600,000 to 9,000,000 per cu.mm., but by the tenth week, the hemoglobin had dropped back down to 12.2 grams per 100 cc. of blood, showing a rise of 7.6 grams of hemoglobin for the ten weeks. The hemoglobin curve leveled out at this point. The red blood

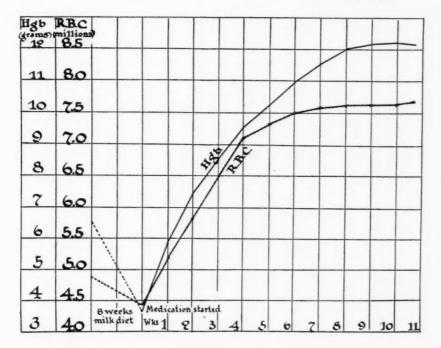
count showed a similar drop at the end of the ten weeks to 8,450,000 cells per cu.mm.

Still another group was placed on 2 min. of the sodium arsenite solution in the same proportion as used in Arsenoferratose per rat per day. During the first two weeks of treatment there was a rise of about one-half gram of hemoglobin and an increase of 750,000 red blood cells. This quickly fell back and continued to fall until the animals died, about the twenty-fourth week. This

Arseno-Ferratose

Chart II

2 min daily per rat 2 min = 0.47 Mgm.Fe



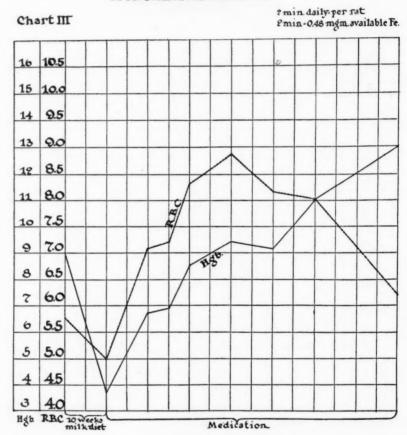
group lived longer than did the control group but their hemoglobin and red blood cell picture was essentially the same.

A group was put on Arsenoferratose with Copper 2 min. per rat per day. The blood changes here were essentially the same as those with Arsenoferratose or Ferratose alone. These were receiving 0.47 mgm. of iron and 0.0037 mgm. of copper daily.

One group of rats with an average hemoglobin of 3.6 grams per 100 cc. of blood was placed on 2 min. of a solution of iron and ammonium citrate contain-

ing 3840 mgm. (60 grains) of the iron and ammonium citrate to the ounce. Each rat was therefore receiving approximately 17 mgm. of the salt or 2.9 mgm. of free iron daily. At the end of three weeks the average hemoglobin for the group was 11.1 grams per 100 cc. of blood, and the red blood count had jumped from 4,600,000 to 7,850,000. The animals were kept on this dosage

GROUP A
Iron Ammonium Citrate



and four and one-half weeks later the hemoglobin was 11.3 grams per 100 cc and the red blood count was 8,400,000. It should be noted that this group was receiving over 6 times the amount of available iron as the above groups.

A group of animals was placed on iron and ammonium citrate so that they received 0.48 mgm. of iron daily. In ten weeks these showed an increase of 7.3

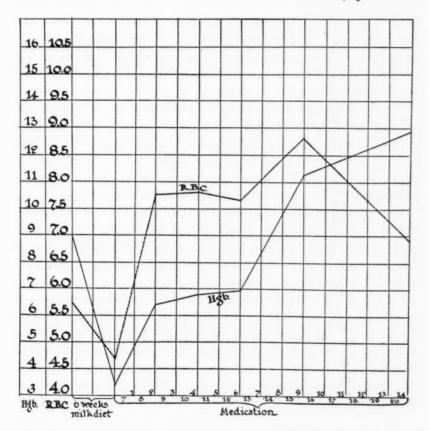
grams of hemoglobin and at the end of fourteen weeks the rise had increased to 9.3 grams, with a corresponding rise in the red blood count (chart 3).

The group on iron pyrophosphate, receiving 0.47 mgm. of iron daily showed an increase of 8.2 grams of hemoglobin in ten weeks (chart 4). A group on the

GROUP D Iron Pyro Phosphate

Chart IV.

2 min. daily per rat 2 min. -0.47 Mgm available Fe.



same drug but receiving only 0.18 mgm. of iron showed an increase of hemoglobin of 4.7 grams in ten weeks and 8.9 grams increase in fourteen weeks.

Another control group was put back on a regular standard stock diet when their hemoglobin averaged 5.5 grams per 100 cc. and with a red blood count of 4,980,000. No medication was administered. At the end of $5\frac{1}{2}$ weeks, their hemoglobin was 13.4 grams per 100 cc. or an increase of 7.9 grams per 100 cc. of blood. During this time, the red blood count rose to 7,410,000 or an increase of 2,430,000 red blood cells.

SUMMARY OF EXPERIMENTAL DATA

The optimum daily dose of iron for the best consistent results in the anemic albino rat was found to be 0.47 mgm. This corresponds closely with the 0.5 mgm. of iron used as the optimum dose for the albino rat by Beard and Meyers⁶ and 0.4 mgm. per rat used by Mitchell and Schmidt.⁷ Our findings in general were in accord with the work of the above investigators as well as with that of Mitchell and Schmidt,⁷ Drabkin and Waggoner⁸ and Keil and Nelson.⁹

Of the different products used in the experiment, Arsenoferratose was found to produce the greatest and most constant rise in hemoglobin. An average increase of 8.4 grams of hemoglobin per 100 cc. of blood was obtained in ten weeks with the 0.47 mgm. daily dose. On a dose containing 0.18 mgm. it required approximately fourteen weeks to reach this point.

Iron pyrophosphate showed the next best effect upon hemoglobin with an increase of 8.2 grams hemoglobin per 100 cc. blood in ten weeks on the optimum dose. The smaller dose of 0.18 mgm. of iron daily produced essentially the same results as with the smaller dose of Arsenoferratose.

Iron and Ammonium citrate showed an increase of 7.3 grams of hemoglobin per 100 cc. for ten weeks' treatment. Iron in the form of Ferratose (elixir bi-ferratin) produced an increase of 7.6 grams of hemoglobin in ten weeks, while Arsenoferratose with Copper produced 7.65 grams hemoglobin in ten weeks. The hemoglobin remained at about 12.5 grams per 100 cc. after this time.

As a supplement to the experimental work just described, we studied a series of patients on the same drugs and their component parts. Patients from the Obstetrical Clinics were chosen

for this work, as we were interested in following any effect on the babies.

Seventy consecutive patients coming to the Temple University Prenatal Clinic, whose hemoglobin was eleven grams per 100 cc. or below and who showed no obvious cause for their secondary anemia, such as bleeding hemorrhoids, vaginal bleeding, bleeding gums, etc., were selected. These constituted the average run of clinic patients. They were receiving no other form of medication during the period in which they were attending the clinic and remained on their usual diet, which in many cases was none too good. Those patients were first seen in the clinic on the average about 19 weeks before their delivery. Of the group, all but 8 were followed during their pregnancy and for at least a month afterward. Fifty of the babies were followed for a period of at least two months. The majority of the patients were delivered in the hospital; a few were delivered at home, by either members of the staff or students.

The patients were divided into 7 groups of 10 each. Each group received a certain type of medication and the hemoglobin estimations and red blood counts were made every two weeks. Arsenoferratose was used for the greater part of the experiment, and was given to the first group in 1 teaspoonful-doses, three times daily. These patients were, therefore, receiving about 48 mgm. of available iron daily, and 0.48 mgm. of metallic arsenic.

A second group was given Ferratose also corresponding to 48 mgm. of available iron daily. A third group was given the vehicle to which sodium arsenite was added, so that they received 0.48 mgm. of the arsenous oxide daily. A fourth group was given the vehicle alone, receiving 1 dram three times daily. A fifth group was given Arsenoferratose with Copper, equivalent to a daily intake of 48 mgm. of iron and approximately 2 mgm. of copper. A sixth group was given iron and ammonium citrate, made up in the vehicle of Arsenoferratose with 60 grains to the ounce. These patients were receiving about 325 mgm. of free iron daily. Therefore, this group was receiving over 6 times the amount of free iron as the Ferratose group.

Finally a control group was used, which received no medication at all during the time they were observed. The average red blood counts and hemoglobin estimations for each of of these groups are given in table 1. The patients on iron and ammonium citrate at the time of delivery showed an increase of 0.77 per cent of hemoglobin, and 0.06 per cent red blood cells over the original estimation, while five weeks after delivery they showed 2.02 per cent average increase in hemoglobin over the original and 0.12 per cent in red blood cells.

Those on Arsenoferratose at time of delivery showed an increase of 1.52 per cent hemoglobin and 0.08 per cent red blood cells, and at the end of 6 weeks they showed an average increase of 1.83 per cent of hemoglobin and 0.12 per cent in red blood cells.

The group on Ferratose alone showed a loss of 0.14 per cent of the original hemoglobin and also a loss of 0.008 per cent of their original red blood cells. At the end of five and a half weeks they showed 0.19 per cent average increase in hemoglobin and 0.09 per cent increase in red blood cells.

The group on sodium arsenite showed essentially no change throughout. The vehicle of Arsenoferratose alone produced an increase of 1.62 per cent of hemoglobin at time of delivery with a loss of 0.015 per cent of the original red blood cells. Five weeks after delivery, there was a 2.93 per cent increase in the hemoglobin and 0.77 per cent increase in red blood cells.

Arsenoferratose with Copper showed a 0.95 per cent increase in hemoglobin and 0.007 per cent loss in red blood cells at delivery, and 1.32 per cent increase

TABLE 1
EFFECT OF IRON THERAPY UPON ANEMIC PREGNANT WOMEN

MEDICATION	NUMBER OF PATIENTS FOLLOWED	ORIGINAL HEMOGLO- BIN AVERAGE	ORIGINAL R.B.C. AVERAGE MILL.	AVERAGE WEEES TREATED BEFORE DELIVERY	EEMOGLOBIN AT DE- LIVERT AVERAGE	B.B.C. AT DELIVERY AVERAGE MILL.	AVERAGE WEEKS FOLLOWED AFTER DELIVERY	FINAL HEMOGLOBIN AVERAGE	FINAL B.B.C. AVER- AGE MILL.	HEMOGLOBIN GAIN LOSS AT DELIVERY	R.B.C. GAIN LOSS AT DELIVERY
		grams			grams			grams		per cent	per cent
Iron ammonium											
citrate	10	10.4	3.63	19	11.2	3.92	5.2	12.5	4.07	+0.77	+0.08
Arseno-ferratose.	8	9.8	3.58	15.5	11.2	3.87	6.0	11.5	4.01	+1.52	+0.08
Ferratose	7	10.5	3.69	20.0	10.4	3.66	5.5	10.7	4.03	-0.14	-0.01
Sodium arsenite.	10	10.0	3.72	15.3	10.2	3.68	5.6	10.1	3.70	+0.02	-0.01
Vehicle	8	9.3	3.93	18.0	10.9	3.87	5.0	11.9	4.20	+1.62	-0.02
Arseno-ferratose											
copper	9	10.8	4.08	18.1	11.8	4.05	8.5	12.2	4.31	+0.95	-0.01
Control	10	10.5	3.77	17.2	10.7	3.75	5.8	10.5	4.13	+0.18	-0.01

in hemoglobin and 0.056 per cent red blood cell increase at the end of the 2 months following delivery.

The control group showed an 0.18 per cent increase in the hemoglobin, while the red blood cells showed essentially no change. About six weeks following delivery the hemoglobin was at the exact point as the original hemoglobin, while the red blood cells showed a slight increase of 0.097 per cent.

Fifty babies were followed for a period of two months. A few stillbirths and early deaths reduced the number of babies followed below the number of women patients listed above. The living conditions of many of the patients were far from desirable.

The babies whose mothers had received Arsenoferratose retained more than 75 per cent of their original hemoglobin at the end of 2 months. The babies whose mothers had received iron and ammonium citrate retained 67 per cent,

Ferratose 68 per cent, Arsenoferratose with Copper 56 per cent, sodium arsenite 69 per cent, and those of vehicle alone retained 71 per cent of their original hemoglobin at birth.

The babies of the control group of mothers retained only 63 per cent of their original hemoglobin. The entire group of babies averaged 17.02 grams of hemoglobin at birth as compared to the mean normal value of blood hemoglobin of 23.2 grams at birth according to Cameron and Nicholson (13). Their normal drop at the end of 2 months was down to 16.2 grams per 100 cc. or 70 per cent of the original hemoglobin. The various blood findings on these babies are shown in table 2.

TABLE 2

EFFECT UPON THE HEMOGLOBIN AND RED CELL COUNT OF THE INFANT AFTER BIRTH PRODUCED BY ADMINISTRATION OF IRON DURING PREGNANCY TO ANEMIC EXPECTANT MOTHERS

MEDICATION RECEIVED BY MOTHER	NUMBER OF BABIES FOLLOWED		R.B.C. AT BIRTH AVERAGE MILL.	FINAL HEMOGLO- BIN AT 8 WEEKS	FINAL R.B.C. AT 8 WEEKS	ORIGINAL HEMOGLO- BIN RE- TAINED 8 WEEKS	ORIGINAL R.B.C. RE- TAINED 8 WEEKS
		grams				per cent	per cent
Iron ammonium							
citrate	9	17.5	5.48	11.8	4.15	67.4	75.6
Arseno-ferratose	5	17.8	5.66	13.5	4.49	75.9	79.4
Ferratose	6	17.0	5.45	11.7	3.98	68.8	73.2
Sodium arsenite	9	15.5	5.11	10.7	3.94	69.0	77.1
Vehicle	8	16.1	5.33	11.5	4.13	71.7	77.5
Arseno-ferratose							
copper	4	17.5	6.02	9.9	3.66	56.6	60.8
Control	1	17.8	5.58	11.3	4.04	63.4	72.4

SUMMARY OF CLINICAL RESULTS

It is obviously rather difficult to interpret the clinical data as there was no practical means of controlling the patient's diet from the standpoint of daily iron intake. It is noteworthy, however, that Ferratose, Arsenoferratose, and Arsenoferratose with Copper gave clinical results comparable to those which were obtained by the administration of iron and ammonium citrate in a much higher dosage. Even the Arsenoferratose vehicle alone, because of its stomachic qualities, resulted in a rise in hemoglobin and red cell count equivalent to that observed in patients receiving iron therapy. Patients receiving no medi-

cation showed little improvement in the blood picture during the observation period. This indicates that active iron therapy is of value in the anemias of pregnancy, and even an improvement in appetite is of great assistance in maintaining adequate iron intake through the normal diet.

CONCLUSIONS

Several commonly prescribed iron preparations have been studied experimentally and clinically for their effectiveness in the treatment and prevention of nutritional anemias. These studies embrace observations on 300 young white rats in a carefully controlled series of experiments, and also the comparative effect of various preparations of iron on the blood picture of expectant mothers as well as that of the child following delivery. All preparations were given in the average recommended dosage for each.

It would appear that neither the rise in hemoglobin nor the increase in red cells is proportional to the amount of iron daily ingested. "Bi-Ferratin," the iron constituent of Arsenoferratose and Ferratose, exerted essentially the same hematinic effect in anemic patients as did iron and ammonium citrate, even though the daily quantity of iron administered was but $\frac{1}{6}$ of that contained in the latter compound. In the experimental animals, however, while massive doses of iron and ammonium citrate gave a more rapid rise in hemoglobin and erythrocytes, this rise was not proportional to the amount of iron administered.

Copper did not seem to enhance the value of iron either in the experimental animals or in patients. This observation, however, is of little significance in view of the fact that the diet in patients was not controlled with respect to this element.

Arsenic exerted little effect upon hemoglobin formation or upon hematopoiesis when employed alone, or in combination with iron in experimental animals. Arsenoferratose, however, gave a more rapid and constant hemoglobin response than any of the other preparations which were employed in the clinical studies. The effect of this element is, therefore, probably alterative and stomachic in nature. The Arsenoferratose vehicle which was used as a control was without effect upon the course of experimental anemia, but clinically its stomachic properties resulted in a striking improvement in the appetite of both mother and child. It was interesting to note that the change in blood picture was equivalent to that produced by iron and ammonium citrate or any of the other preparations of iron. This we attribute to increased ingestion of food.

The necessity for administering massive doses of ionizable iron in the treatment of nutritional anemias is not apparent, when smaller doses of some more palatable preparation will cause practically the same rise in hemoglobin and erythrocytes without producing undesirable side-effects.

The response of animals, in a well controlled series of experiments, to the administration of iron may be essentially different from that of patients under actual clinical observation, and hence the interpretation of such experimental data should be made with the utmost conservatism.

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A STUDY OF THE COMPLEMENT FIXATION REACTION IN TUBERCULOSIS*

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During the past year the Committee on the Evaluation of Serodiagonstic Tests for Syphilis of the United States Public Health Service, coöperating with the American Society of Clinical Pathologists,† conducted a study bearing upon the specificity of the serum tests for syphilis in tuberculosis.

One of us (J. A. K.) was a participating serologist conducting the Kolmer complement-fixation test for syphilis with the specimens of blood received in this survey.

With a large number of these there were sufficient sera left over for the conduct of the tuberculosis complement-fixation test and since the donors were selected with great care and the tests conducted with specimens bearing a number only with no knowledge of the clinical status of the donors, it was thought advisable for one of us (J. A. K.) to conduct these additional tests and routinely submit reports on the results (to J. F. M.).

SELECTION OF DONORS

408 sera from tuberculous donors were available for the tuberculosis complement fixation tests. These were contributed by nine tuberculosis sanatoria.‡

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[†] Thomas Parran, Surgeon General, U. S. Public Health Service, Chairman. H. H. Hazen, M.D., Washington, D. C. J. F. Mahoney, Sr. Surgeon, U. S. Public Health Service. Arthur H. Sanford, M.D., Rochester, Minn. F. E. Senear, M.D., Chicago, Ill. Walter M. Simpson, M.D., Dayton, Ohio. R. A. Vonderlehr, Assistant Surgeon General, U. S. Public Health Service.

[‡] Bellevue Hospital; New York City (Dr. J. Burns Amberson, Jr.). Herman Kiefer Hospital; Detroit, Michigan (Dr. Bruce N. Douglas, Tuberculosis

All the donors presented from moderately advanced to far advanced tuberculosis. Most of them showed tubercle bacilli in the sputum and approximately 50 per cent were classified as febrile. The presence of syphilis was excluded from this group as completely as possible by history and physical examination. About half were females.

As previously stated, specimens were submitted by code letter and number only, the laboratory being entirely unaware of the clinical status of the donors.

As originally intended, each sanatorium was to include indiscriminately a group of specimens from patients with known syphilis, without coexisting pulmonary tuberculosis. Sera from 37 syphilitic individuals were available for these tuberculosis complement-fixation tests for the purpose of determining the number or percentage giving positive reactions through the fixation of complement by syphilis antibody and the lipoids present in the tuberculosis antigen. But because of the lack of access in some of the sanatoria to donors with syphilis only, it became necessary to include some with dual infections (tuberculosis and syphilis).

METHOD EMPLOYED

As is well known, many methods have been advocated for the preparation of antigen of tubercle bacilli for the complement-fixation test. In a study of antigens prepared according to 22 methods Kolmer¹ found that all were capable of giving some positive reactions with the sera of syphilitic nontuberculous individuals, presumably due to the fixation of complement by syphilis antibody and the lipoids and waxes of the bacilli. Antigens prepared of degreased bacilli were found to be much less likely to give these cross complement-fixation reactions but, even when extracted with various lipoid solvents to the point of losing acid-fastness, antigens of the residues were found to still possess a tendency to cross reactions with syphilis antibody although not nearly to the degree observed with antigens of whole bacilli. Furthermore, antigens of degreased bacilli were found to be less antigenic in tests with tuberculosis antibody indicating that the lipoids of tubercle bacilli play some part in the fixation of complement by tuberculosis antibody.

Controller). Michigan State Sanatorium; Howell, Michigan (Dr. Geo. L. Leslie, Medical Supt.). Robert Koch Hospital; Koch, Missouri (Dr. G. D. Kettelkemp, Supt.). Sea View Hospital; West New Brighton, Staten Island, N. Y. (Dr. Geo. O. Ornstein). Tuberculosis League of Pittsburgh; Pittsburgh, Pa. (Dr. C. Howard Marcy). Municipal Tuberculosis Sanatorium; Chicago, Ill. (Dr. Leo M. Czaja, Gen. Supt.). Wm. H. Maybury Sanatorium; Detroit, Michigan (Dr. Bruce H. Douglas, Tuberculosis Controller). The North Carolina Sanatorium for the Treatment of Tuberculosis; Sanatorium, N. C. (Dr. P. P. McCain, Supt.).

However, it appears advisable to prepare antigen of at least partly degreased bacilli and that employed in the present study was prepared of a human strain about eight years ago according to the method of Kolmer² as follows:

1. The strain was cultivated in glycerin broth for four weeks and autoclaved at 10 pounds pressure for twenty minutes.

2. The killed culture was passed through several layers of filter paper and the residue washed repeatedly with distilled water for the removal of glycerin.

3. The residue was dried in a desiccator over sulphuric acid.

4. The bacillary residue was finely ground in a ball mill and 1 gram added to 200 cc. of ether followed by boiling one hour with a reflux condenser. The ether was discarded, the residue dried and boiled with 200 cc. of acetone. The acetone was discarded, the residue dried and boiled again with 200 cc. of absolute ethyl alcohol for an hour. The alcohol was discarded, the residue dried and added to 190 cc. of distilled water followed by boiling with a reflux condenser for three hours. To this was added 2 grams of sodium chloride for isotonicity and 10 cc. of 5 per cent phenol as a preservative.

This antigen was titrated for anticomplementary activity and used in the complement-fixation tests in dose corresponding to one-third of the anticomple-

mentary unit.

Natural antisheep hemolysin was removed from each serum by absorption with washed sheep corpuscles. Each serum was then heated at 55°C. for 20 minutes and tested in amounts of 0.2, 0.1, 0.05, 0.025 and 0.005 cc. with 0.2 cc. in the serum control with a primary incubation of two hours in a water bath at 37°C., the balance of the test and readings being according to the technic of the Kolmer complement-fixation test for bacterial diseases.³

In view of the age of the antigen employed it is likely that some antigenic sensitiveness was lost but we have no data directly bearing upon this subject with the particular antigen employed.

RESULTS

As shown in table 1, of 270 sera from far advanced cases of pulmonary tuberculosis 179, or 66.3 per cent, gave positive reactions; of 137 sera from moderately advanced cases 81, or 51.8 per cent, gave positive reactions. The serum of one case of osseous tuberculosis gave a negative reaction. Of the total 408 sera therefore 260, or 63.7 per cent, yielded positive complement-fixation reactions.

Records were available in 397 of the donors on the presence or absence of fever at the time the specimens of blood were secured. As shown in table 2, this factor had little or no influence upon the complement-fixation reactions since 85 or 63 per cent of 135 febrile cases gave positive reactions while 168 or 64.1 per cent of 262 afebrile cases reacted positively.

In a previous study by one of us (J. A. K.) with Callahan⁴ employing this type of antigen in tests of 112 tuberculous individuals, positive reactions were observed in 66 to 81 per cent; Kilduffe⁵ reported 64 per cent positive reactions in 104 cases of pulmonary tuberculosis employing the same type of antigen and

TABLE 1

RESULTS OF KOLMER TUBERCULOSIS COMPLEMENT FIXATION TESTS WITH THE SERA OF 408 NON-SYPHILITIC TUBERCULOUS DONORS

CLASSIFICATION	TOTAL TESTED	POSITIVE BEACTIONS	NEGATIVE REACTIONS	PER CENT POSITIVE REACTIONS
Far advanced	270	179	91	66.3
Moderately advanced	137	81	56	51.8
Osseous	1	0	1	

TABLE 2
Influence of Fever on the Kolmer Tuberculosis Complement
Fixation Reaction

CLASSIFICATION	TOTAL TESTED	POSITIVE REACTIONS	NEGATIVE REACTIONS	PER CENT POSITIVE REACTIONS
Far advanced febrile	102	66	36	64.7
Far advanced afebrile	159	108	51	67.9
Moderate advanced febrile	32	19	13	59.4
Moderate advanced afebrile.	103	60	43	58.2
Osseous febrile	1	0	1	

technic, while Ogawa⁶ reported 79 per cent positive reactions in a group of 263 cases of pulmonary tuberculosis of varying duration and severity. As previously stated, 63.7 per cent positive reactions were observed in this study of 408 sera from tuberculous donors and this somewhat smaller percentage of positive reactions may have been due to some loss in antigenic sensitiveness of the antigen employed due to the fact that it had been prepared about eight years previously.

CROSS COMPLEMENT-FIXATION REACTIONS BY SYPHILITIC SERA AND TUBERCULOSIS ANTIGEN

Of the sera from 37 syphilitic donors 12 were known to be from cases of dual infection with both syphilis and tuberculosis. The remaining 25 were cases regarded and treated as syphilis only without evidence of coexisting tuberculosis but none of these were subjected to exhaustive examination for the absolute exclusion of the latter.

As shown in table 3 eight of the 12 donors with syphilis and

TABLE 3

RESULTS OF SYPHILIS AND TUBERCULOSIS COMPLEMENT FIXATION TESTS WITH THE SERA OF 12 SYPHILITIC AND TUBERCULOUS DONORS

SERA	CLASSIFICATION OF SYPHILIS	REACTIONS WITH SYPHILIS ANTIGEN	REACTIONS WITH TUBERCU- LOSIS ANTIGEN
253T	Primary; treated		
256T	Primary; treated		2
359P	Primary; treated	4 4 2	2
337P	Primary; treated	4 4 2	
347P	Primary; treated		1
362P	Primary; treated	4 4 2	
251T	Primary; untreated		
356P	Primary; untreated	4 2	
357P	Primary; untreated	41	
365P	Secondary; treated	4 4 3	
360P	Tertiary; treated	4 4 3	21
358P	Tertiary; untreated	4 4 3	

tuberculosis gave positive Kolmer complement-fixation reactions with syphilis antigen (C. L.) and 4 gave positive reactions with tuberculosis antigen. Whether or not the latter were due to fixation of complement by syphilis antibody and the lipoids of the tubercle bacillus antigen, or fixation by tuberculosis antibody and tuberculosis antigen, cannot be stated but the latter is possible since all of these donors were known to have dual syphilitic and tuberculous infection.

As shown in table 4, twenty-three of the 25 sera from syphilitic donors presumably free of tuberculous infection gave positive Kolmer complement-fixation reactions with syphilis antigen (C. L.). Since the results of the survey (to be published) showed the Kolmer complement-fixation test to yield no falsely positive reactions with the sera of syphilitic non-tuberculous donors, it is apparent that all of these positive reactions were due to the

TABLE 4

RESULTS OF SYPHILIS AND TUBERCULOSIS COMPLEMENT FIXATION TESTS WITH THE SERA OF 25 SYPHILITIC BUT PRESUMABLY NON-TUBERCULOUS DONORS

SERA	CLASSIFICATION OF SYPHILIS	REACTIONS WITH SYPHILIS ANTIGEN	REACTIONS WITH TUBERCU- LOSIS ANTIGEN	
103P	Primary; treated	3 2		
108P	Primary; treated	21		
110P	Primary; treated	4 4 2		
111P	Primary; treated	2 1		
116P	Primary; treated		21	
118P	Primary; treated	4 4 2		
253P	Primary; treated	4 4 3 1 -		
254P	Primary; treated	4 4 1	2 2	
255P	Primary; treated	4 3 2		
520P	Primary; treated	4 4 3	21	
529P	Primary; treated	4 4 3		
119P	Primary; treated	4 4 2		
540P	Primary; treated	4 4 4 2 -		
543P	Primary; treated			
560P	Primary; treated	4 3		
563P	Primary; treated	4 4 1		
518P	Primary-Secondary; treated	4 4 3	2 1	
519P	Primary-Secondary; treated	4 4 3		
530P	Secondary; treated	4 4 4 1 -	4 4 2	
212P	Tertiary; treated	4 4 2 1 -		
539P	Tertiary; treated	4 4 1		
114P	Tertiary; untreated	4 4 3		
121P	Tertiary; untreated	4 4 2		
112P	Neuro; treated	4 4 2		
552P	Neuro; treated	4 4 2		

fixation of complement by syphilis antibody and syphilis antigen (C. L.).

But 5 of these sera gave positive reactions with the tuberculosis antigen. Since these donors were believed to be free of active tuberculosis the reactions were presumably due to fixation of

complement by syphilis antibody and the lipoids escaping extraction in the preparation of the tuberculosis antigen in four of these sera. With one, however, (116P) the syphilis reaction was negative while the tuberculosis reaction was positive raising the question whether or not this particular donor may not be tuberculous and the positive reaction due to fixation of complement by tuberculosis antibody. If this case is excluded it would appear therefore that 4, or 16 per cent, of the 25 syphilitic non-tuberculous donors gave positive complement-fixation reactions with the lipoids of the tubercle bacilli.

Kilduffe⁷ has reported that about 10 per cent syphilitic but non-tuberculous individuals may give cross or non-specific complement-fixation reactions with the lipoids of tubercle bacilli; our results have been essentially similar and for this reason it is always advisable to conduct the Wassermann test with each serum submitted for the tuberculosis complement-fixation test. If the former yields a positive reaction great caution is required in the interpretation of a positive tuberculosis reaction.⁸

SUMMARY

1. Tuberculosis complement-fixation tests conducted with the sera of 408 tuberculous indivudals yielded 63.7 per cent postive reactions with an eight-year-old antigen of partially degreased human bacilli prepared and tested by the Kolmer method.

2. The presence or absence of fever at the time of collection of blood specimens had little or no influence upon the results since 63 per cent of febrile and 64.1 per cent of afebrile cases

gave positive reactions.

3. Four or 16 per cent of 25 sera from syphilitic non-tuberculous donors gave positive tuberculosis complement-fixation reactions, presumably due to the fixation of complement by syphilis antibody with the lipoids and waxes remaining in the partially degreased bacilli of the tuberculosis antigen.

4. For this reason it is recommended that the Wassermann test be conducted routinely with all sera submitted for the tuber-culosis complement-fixation test; when the former yields a

positive reaction great caution is required in the interpretation of a positive tuberculosis reaction.

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THE CRITERIA OF A DEPENDABLE BASAL METABOLISM REPORT*

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The clinical use of the Basal Metabolism test, marks a new era in medicine. It dates back to the publication, twenty-one years ago, of Standards for the Normal elaborated by Aub and Dubois (1917)¹, and by Harris and Benedict (1919)³. The fundamental data previously accumulated in 1914 and 1915 and which offered the basis for these original and indispensable standards, are given in full on pp. 31–48 of the last mentioned publication. Heretofore, clinical medicine had profited relatively little from the tremendous amount of fundamental research done in the field of body calorimetry for nearly a century by physiologists of Europe and of the United States.

Up to 1917 metabolimetry had remained confined to the experimental laboratory and was under the control of highly trained technicians. According to one of these pioneers, the number of technicians in the United States capable of manipulating successfully the complicated apparatus and technic then in use could be counted on the fingers of one hand. With the subsequent introduction of simpler and less costly equipment and, in particular, of the long hoped for normal standards, clinical metabolimetry rapidly spread the world over.

The ease with which anyone of ordinary intelligence can master the universally adopted technic in conducting a "basal metabolism test" with the newer types of apparatus, resulted in a rapid multiplication of number of operators. Many of these technicians, educated over night merely to run a machine, lacked the

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training and experience necessary to face the multiplicity of problems to be otherwise encountered. The indispensable ability to secure the coöperation of the individual under observation is sadly wanting in the majority of inexperienced operators but the reliability of their work and reports was generally unquestioned because there were few capable of checking up and passing judgment upon it.

High class technicians are no longer indispensable for the manipulation of the type of metabolimetric apparatus now in general use. As a result we have with us a large number of technicians who operate beyond their rightful limitations.

More than one authority has stated, on the basis of extensive travel and observation, that as many as 70 per cent of the basal metabolism reports made today by the average operator may not be worth the paper upon which they are written. Although such a distressing situation calls for a remedy, it is not the slightest wish of the unprejudiced critic to return to the days of not long ago, when only highly trained technicians could be entrusted with the delicate method of gas analysis then and still required by the so called "open circuit" method of body calorimetry. general acceptance of the "closed circuit" type of apparatus did not reduce in any way the dependability and the accuracy of the determination. On the contrary, valuable technical improvements made these newer and simpler facilities better suited for their wide application in clinical medicine. However, the universal adoption of this method has often placed clinical metabolimetry beyond the judicious control desirable for the maintenance of high medical standards. It is regrettable that to a large extent the blame lies at the door of the internist and even of the pathologist.

To come to the point, a plea is here offered to the Society of Clinical Pathologists to extend more definitely its constructive influence in this important branch of pathology as an important, timely remedy for the present state of affairs. It is needless to discuss before this specialized group the harm which results from the misleading information now often accepted without question

from a large number of unapproved or unsponsored laboratories or technicians.

The metabolism operator and his department are too often not under the supervision of an approved clinical laboratory or pathologist. Whether his services are regarded as reliable usually depends merely on his general reputation. It is the purpose of this paper to present criteria by which independent judgment may be passed on the reliability of metabolism tests.

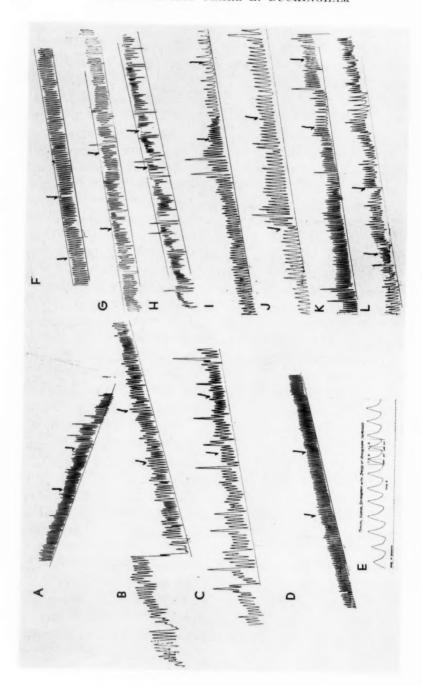
The first essential in reliable metabolism testing is an adequately trained technician, sponsored by a competent director or clinical pathologist. The technician should see that every report includes all the routine notations which are the indispensable earmarks of

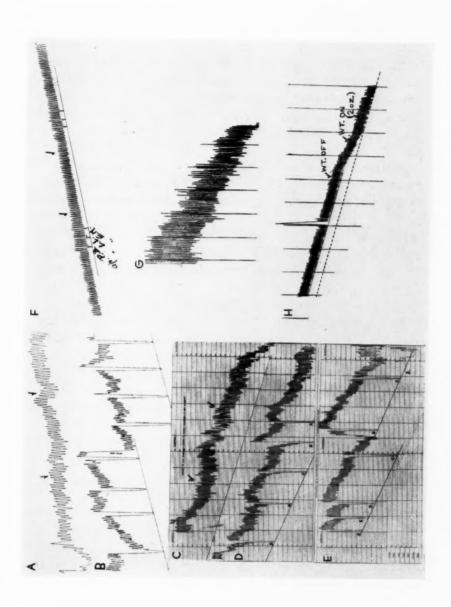
a complete and properly conducted test.

In the use of the "closed circuit" method the kymograph plays an important part not only in simplifying the technic, but also in increasing the accuracy of the spirometric measurements on which are based the determination of the rate of oxygen consumption. More than that, the spirogram is a permanent and trustworthy record of the kind of technic pursued in the performance of the test, as well as of the general behavior or coöperation of the subject.

We have repeatedly had proof of the fact that, in general, little meaning is attached to the spirogram aside from its being a convenient means of measuring oxygen consumption. The spirogram of each determination should accompany the report because it furnishes the most important evidence of a successfully conducted test. In fact, no disturbance which would materially affect the results can occur without leaving some significant telltale in the respiratory tracing. It should be preserved as a permanent record, either in the laboratory or in the doctor's files.

With the spirogram and certain other information which will be mentioned, one can verify to his own satisfaction the dependability and accuracy of any metabolism test. One does not need to have observed the test, even in part. Such verification, of course, might not detect sheer dishonesty and intentional





fraudulent action on the part of the operator. But the simple check-up here proposed should at least reveal possible carelessness and ineptitude. If this check is routinely imposed as an every day rule in the laboratory, it will act as a persistent spur in inculcating habits of carefulness and accuracy in the work of the technicians.

We are not introducing a new technic. It is fundamentally the same as that introduced by one of us⁵, in 1922, and now universally adapted to all different makes of the Benedict type of metabolism apparatus. A discussion of their differential features is not necessary because the plan here suggested can be followed in the use of any type of apparatus with little modification.

It may not be sufficiently appreciated how much information can be obtained from the spirogram in addition to the rate of oxygen consumption. Here are enumerated the most important items:

1. The spirogram gives good evidence that the subject has, in all probability, reached the required standard or basal state and maintained the proper degree of relaxation necessary for the accuracy of the test. If, as is often the case, this state of relaxation has been temporarily disturbed by the process of connecting the subject to the apparatus, the respiration tracing will show a steeper trend than that which it will assume later, barring any other disturbance which may again increase oxygen consumption (fig. 1, A). Similar disturbances of a material degree may occur during any portion of the test, as, for instance, towards the end of a too prolonged ordeal, which is always likely to become fatiguing. Any disturbance which may alter the trend or slope of the tracing as a whole must be avoided. Nevertheless, the tendency is toward a too short rather than a too prolonged test period. Ten minutes is a good average.

In types of apparatus which will allow it, a tracing which shows considerable irregularity can be given a fresh start by merely adding oxygen while the patient continues to breathe into the apparatus (fig. 1, B).

We deem it indispensable to have a proof of the absence of a

leak during the test. Fig. 1, B, as well as several others, gives this evidence. We know of no other satisfactory test for leak than this simple one which is performed by placing on the spirometer bell, for a period of one and one-half or two minutes, (at about midway through the test) a weight of not over two ounces. A steeper slope of the spirogram while the weight is on is a positive indication of a leak (fig. 2, H).

2. The oxygen consumption line. The slope of this "O2 line," so called, serves for the accurate measure of the oxygen consumed

in a given time during the test.

This O₂ line must be accurately located in the following manner (fig. 1, C): Select that portion of the graph which presents the most uniform trend for about five or six minutes or longer, and draw the O₂ line according to the general trend of the slope of the graph. This line is drawn close to, but preferably under the expiration points, avoiding intersection of the deeper ones which may have been picked out as the very best for the purpose, especially when found distributed more or less periodically throughout the graph. Shorter portions, if particularly steady though less reliable because of their shortness, are not to be ignored entirely if nothing better is available. These short portions may sometimes be more trustworthy than longer ones with an erratic course.

Figure 1, D gives evidence of a satisfactory state and behavior of the patient throughout the test. This is the most frequently encountered type of tracing.

A respiratory pause in normal, quiet breathing takes place at the end of each passive expiratory movement, therefore at the time when the lungs are at a uniform degree of deflation. A peculiar reversal of events is occasionally observed, the graph revealing instead a respiratory pause with a coincident uniform lung inflation at the end of each inspiratory movement. Figure 1, F, G and H shows this rare, "inverted" type of breathing which, incidentally has been found by Nielsen and Roth⁴ to occur almost exclusively in women. In such cases the O₂ line is preferably traced along the inspiration line.

3. Possible errors due to change of lung expansion during a

metabolism test. Little or no stress is laid, as a rule, on the considerable degree of error that can be introduced in metabolimetric measurements by possible changes in the degree of lung expansion during the period selected for measurement (fig. 1, I, J, K, and L). It stands to reason that whether only two or many expiration points are to serve as a basis for the location of the O₂ line, reasonable assurance must be had that at the time of their occurrence a like degree of lung expansion is present. While one can assume that such is the case whenever the selected period or the entire graph presents a uniform appearance from beginning to end, alteration of lung volume at the end of expiration is the most frequent direct cause of irregular shifts in the respiration tracing. It is evident that these shifts are not accompanied by an increased or decreased oxygen absorption during an ordinary basal test, unless at the time the subject is also more or less disturbed.

The "open circuit" method presents some very definite advantages, particularly in conducting certain types of research. We agree, for instance, with the argument of Boothby, Berkson and Dunn² that when using this type of apparatus a possible change in the "reserve" or "supplementary" respiratory volume, in a subject under test, does not vitiate the determination, as can happen during a test made with a closed circuit apparatus. However, the possibility of a change of that nature is rarely encountered during a uniform period of quiet, effortless breathing, as should be insured during any metabolism test whether performed by the closed or open circuit method. We also feel confident that whenever any material change of this nature occurs the graph will reveal it, and efforts such as are suggested in this paper can be made to preclude any consequent error.

4. Methods of retaining deep expiration points when desired. Figure 2, A and C illustrates a type of tracing which is very unsatisfactory and even useless. In most cases a satisfactory spirogram will usually be obtained after one or more tests on the same or on a subsequent day. Stubborn cases of erratic breathers are seldom encountered. They are often difficult or impossible to account for and may be found in perfectly normal subjects. They generally can be trained to be fairly regular breathers, provided several days can be spent in the attempt.

Method A. By Voluntary Deep and Full Expiration Only. In one of our most refractory cases we contrived and successfully applied the following method of obtaining, at will, deep expiration points for the location of the O₂ line. Before the test starts, the patient is told that near the beginning and again near the end, he will be asked to exhale as deeply as possible, and that he is to do so only after having taken in an ordinary, normal breath. The signal should not be given unless there is sufficient capacity for the additional exhaled air in the spirometer. In other words, sufficient space must be allowed for the pen to trace a full expiration without running off the paper (fig. 2, B). This tracing also illustrates how this was accomplished successfully in a typical case in which no satisfactory check could be obtained after several training attempts.

Method B. By Voluntary Deep and Full Expiration, with Manual Compression (by Operator) Over Costal Margins. This modification of Method A is usually advisable because many subjects cannot be trusted to exhale with the same degree of effort and completeness at the beginning and at the end of the test. The difficulty can readily be overcome by the simple manual application of pressure over the costal margins, by the operator, at the time the subject is asked to "breathe out completely." In doing so, a still deeper, and more uniform deflation of the lungs is obtained. Graphs show, as illustrated in figure 2, D and E that a remarkably constant degree of deflation is registered though variable but sufficient compression force is applied. This is well shown in figure 2, F which shows the tracing obtained when manual chest compression over the costal margins was applied in succession by three operators of different muscular strength.

The choice between Method A and Method B will depend on the degree of intelligent coöperation which may be expected from the patient. The technician will do well to try one or both of these methods, and to be prepared to apply them without hesitation when necessary. Though they may seldom be needed, they may sometimes be of valuable service.

5. Technical objections to relying on results obtained from the first metabolism test a given patient may have had. If a patient has never had a metabolism test before, we believe that there is

enough chance of unreliable results to warrant taking a second test on a different day with rare exceptions, or unless absolutely impossible. Our experience shows that for clinical purposes subjects who have had the experience of even a single metabolism test may thereafter be considered "trained" even if they have no further tests for years. This may not be true if their first test was poorly conducted.

Whenever possible, we follow a patient's first test with another on the following or a closely succeeding day. During this second test, before the patient is allowed to arise from the couch or even while the test is still in progress, we find it easy to roughly measure the approximate fall of the spirometer bell in six minutes. We thus determine whether the results of this second test will probably check within the required five per cent of the first. If it does not, we immediately obtain another tracing before dismissing the patient, often without removing the mouthpiece.

In a study of 2000 patients having the test for the first time, we found that the first tracing of the second day's test checked with the first day's test, within the required five per cent, in 63 per cent of our cases. In 28 per cent of the cases, the third tracing (that is, the second tracing during the second day's test) afforded the desired check. In $4\frac{1}{2}$ per cent of the cases, they were asked to return for further study in a day or two, when we were able to obtain good results. A final $4\frac{1}{2}$ per cent of cases, though not presenting the usual desirable five per cent agreement, but differences of six to eight per cent instead, were not asked to return for further checking because they were, as a rule, unusually difficult to deal with, and also because they all happened to have rates within the accepted normal range.

The most significant fact revealed in another study of 1000 successive and uninitiated patients, is that without repeated determinations eight per cent of the cases would have been definitely wrongly diagnosed. That is, $4\frac{1}{2}$ per cent would have been classed above the normal (over +10 per cent) when they proved later to be within the normal range, and $3\frac{1}{2}$ per cent would have been classed as normal when they were below the lower limit of the normal range, or below -10 per cent. These statistics are based on the metabolic rates as computed by the Harris-Benedict

normal standards. Were these same 1000 determinations based on the Dubois normals or their later modifications, including the Mayo Foundation Standards, a larger proportion would have been included in the group classed below the normal range.

6. Clinical and physiological metabolimetric standards. There are two schools of thought with regard to normal standards in basal metabolimetry. One supports the so-called "clinical standards," the other, what may be termed "physiological standards." The clinical standards are based on data collected chiefly from first tests on supposedly normal subjects. As is well known, first tests, even with well relaxed subjects, tend to have somewhat higher results than are obtained when the subject has had more or less training. These standards, therefore, tend to have values somewhat higher than those of the physiological standards, which are based on the lower range of values obtained in a series of tests on the same subject.

In interpreting metabolism tests by one or another standard, it is perhaps less important to use a particular standard than it is to understand which type of standard one is using. Since the clinical standards have higher values, the metabolic rates computed by them will tend to run lower than if the physiological standards are used. They will be fairly reliable for first tests, and with untrained subjects. But when a subject has become trained or accustomed to metabolism tests, allowance will have to be made for this factor.

On the other hand, when interpreting tests by physiological standards, the results will tend to be high for untrained subjects, and to be more reliable when the subjects have had a certain amount of training. That is, allowance must be made for lack of training, when using the physiological standards.

For the sake of emphasis, I will say again that the choice of the standard itself is probably less important than knowing which type it is, and, therefore, what allowance must be made in its application under various conditions. We place our main reliance in the physiological standards although we generally report tests on the basis of several standards in our laboratory.

The reason for this choice lies in the fact that the patients who have to be given repeated tests, in the course of checking up the

results of therapy, form the largest and also the most important group. Since they are, by virtue of repeated tests, trained subjects, the physiological standards seem the most logical choice for routine use.

The most commonly used clinical standards have been the Boothby-Sandiford modification of the Dubois normals, though these are superseded now by the new Mayo Foundation Standards. The Harris-Benedict normal standards are the physiological standards most frequently used in this country.

CONDENSED SUMMARY OF ESSENTIALS FOR THE FINAL APPROVAL OF A METABOLISM REPORT

- 1. A trustworthy technician or approved operator.
- 2. Neatness of both the spirogram and the report.
- 3. A carefully located oxygen consumption line.
- 4. Evidence of a negative test for leak in the spirogram.
- 5. Evidence that the subject was relaxed; at physical rest and breathing quietly, at least during the portion of the graph which has served as a basis for the O_2 line.
- 6. Evidence of the efficiency of the soda lime in maintaining the air inspired by the subject CO₂ free (fig. 2, G).
- 7. Temperature of the patient: Subtracting from the B.M.R. 7 per cent for each degree F. rise of body temperature above the normal.

Subnormal body temperature is a different phenomenon and should not be corrected for.

- 8. Pulse rate: An increase during the test of more than a few counts denotes some cause of disturbance.
- 9. Respiration rate: Labored breathing or excessive ventilation indicates either a poorly conditioned apparatus, or sometimes an unrelaxed or uneasy patient, who, as a rule, can easily be induced to do better without interrupting the test.
 - 10. Behavior of the patient.
 - 11. Type of apparatus used.
- 12. Normal standards: The standards upon which the rate is reported should always be mentioned, especially for children. Occasionally it may be very helpful to the clinician to have rates calculated on the basis of two or more applicable normal standards.

The above condensed summary might well be the basis of a mutual agreement among the following parties concerned in the elaboration and the clinical application of a metabolism report:

First: The clinical pathologist, or anyone acting as a medical consultant or expert.

Second: The laboratory director whose duty is to systematically check and endorse the work of technicians under his supervision.

Third: Any physician who may have to assume the duty of approving a report when this responsibility is not shouldered by at least one of the other parties here mentioned.

Fourth: The technician, who should clearly understand his duties and what should be included in a metabolism report to be acceptable for final inspection and approval.

SUMMARY

1. A plea is offered to the Society of Clinical Pathologists to extend its constructive influence for the maintenance of high standards in metabolimetry.

2. Valuable information can be obtained from a comprehensive spirogram in addition to the rate of oxygen consumption obtained from an accurately located O₂ line.

3. Possible errors due to alterations in the subject's lung expansion during a metabolism test.

4. Deep expiration points are of particular value in locating the O₂ line. Methods of obtaining them at will and at any desired moment, with or without manual compression over the costal margins, are described.

5. Technical objections to relying on results obtained from "first" metabolism tests are given. A subject may usually be considered "trained" after having had one properly conducted test. Clinical versus physiological normal standards. We place chief reliance on physiological standards.

6. Condensed summary of essentials for the final approval of a metabolism report for the use, in particular, of the clinical pathologist, laboratory director, and physician.

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THE GOITRE CYCLE AND ITS ANATOMIC FINDINGS*

A REPORT OF 1028 SECTIONED GOITRES

B. MARKOWITZ

During the past thirty years vast contributions have been made to the study of the etiology of goitre. These contributions have done much to bring about very striking changes in the prevention of this disease. Much of this has come about through studies of the anatomic changes found in the thyroid gland in relation to iodine content, and the relation between the thyroid and other glands of internal secretion. While the iodine content and the whole of thyroid chemistry is of extreme importance in preventing goitre, the anatomic changes in the gland are a constant indicator of the course and intensity of existing thyroid disturbance. True, these anatomic changes may reflect only the imbalance present in the gland but they are the only constant findings, and are described as the "hyperplasia-involution cycle."

In a study of over 1,000 operated goitre patients, the correlation of the case histories and the anatomic findings of the surgically removed specimens definitely indicates the existence of such a cycle. Following iodine medication the smooth hyperplastic goitre presents areas of circumscribed colloid which are definite signs of involution. In the long-standing cases with histories of remissions and exacerbations of clinical symptoms, the presence of colloid nodules in various stages of development and degeneration testify to the hyperplasia-involution cycle.

The morphological changes seen in the hyperplasia of puberty, of pregnancy, of simple goitre, in "hyperplastic goitre" and in compensatory hyperplasia following partial thyroidectomy, are all essentially the same. This is contrary to the opinion of Aschoff¹ and McCarrison² but is generally accepted by most workers (Jaffé,² Hertzler,⁴ Marine⁵). The individual thyroid follicle, as first explained by Williams and Pease,⁶ and later modified by Rienhoff,⁵ is the histologic unit of the thyroid gland

^{*} Received for publication, March 24th, 1938.

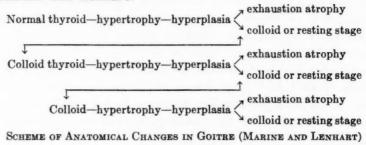
and the cells of this follicle seem to be the essential factor in the hyperplasia-involution cycle.

Whenever the thyroid secretion becomes insufficient, whether due to physiological demand or due to decreased iodine, hypertrophy and hyperplasia occur; the cells of the follicles become larger and taller, they increase in number even to the point of papillary infolding, new follicles are formed and the stainable colloid is replaced.

Whatever the cause, this hyperplasia is apparently due to stimulation by the thyrotropic hormone of the anterior pituitary. Loeb, Leo and Bassett⁸ injected this hormone into the guineapig and produced a similar hyperplasia. When the stimulation of this thyrotropic hormone is decreased either by increased iodine intake or decreased physiological demand or some undetermined cause, the hyperplasia recedes and involution occurs.

The anatomic changes in involution are the reverse of those which occur during hyperplasia; the cells of the follicles become smaller and decrease in number, the blood supply diminishes, and colloid accumulates, filling and distending the follicles. With repetition of this hyperplasia-involution cycle the gland is capable of undergoing hyperplasia and involution many times. The literature calls attention to the probable explanation that the degree of stimulation may affect the resulting hyperplasia-involution cycle.

The degree of anatomical change depends upon the degree of stimulation with resulting hyperplasia, and the degree of involution with resulting recession. These anatomical changes of the thyroid in goitre are well illustrated by the following scheme of Marine and Lenhart.¹⁰



The process is a constant repetition of these changes, with hyperplasia involuting to colloid goitre and colloid goitre progressing to hyperplasia. These anatomical changes may change their pace at any stage, whether during hyperplasia or involution, and even proceed in the opposite direction. With this capability of under-going hyperplasia and involution many times during its life cycle, we point to the accepted thought that colloid goitre is secondary to previous hyperplasia. That colloid goitre occurs in a normal gland from increased colloid secretion, is in the light of present knowledge irrational. All available evidence indicates that goitre begins as an active hyperplastic process whether the end be exhaustion atrophy or colloid goitre.

If exhaustion atrophy occurs, as is seen principally in goitrous endemic cretins, and occasionally as the end result of diffuse hyperplastic goitre, the anatomic picture is that of desquamation and disintegration replacing the very active hyperplasia. If involution to colloid goitre occurs the anatomic picture is that of increased colloid and inactive follicular cells. In a previous paper¹¹ we have called attention to the two distinct processes of thyroid secretion and colloid accumulation which balance each other in the physiology of the thyroid gland. With an imbalance however there is a disproportion of these two processes with resulting nodular formation. The secondary changes that occur with the production of nodules, so-called adenomas, increase with the duration of the goitre. That is, with the continued repeated cycles these nodules increase in size and number. It is possible that the foetal rest theory of Woelfer¹² can explain the true adenoma which presents only cords of cells with no attempt at follicle formation. However the great majority of the nodules found in our series seemed very closely associated with the thyroid tissue of goitre; they are most likely formed by this hyperplasia-involution cycle which in the very beginning caused the diffuse goitre. Graham's speaks of them as "involuted goitres which have been oscillating between involution and hyperplasia for years."

While the thyrotropic hormone of the anterior pituitary appears to be the important factor in stimulating and producing those anatomic changes in the thyroid gland, there is evidence that the converse is also true¹³; that the thyroid also influences the anterior pituitary especially in its production of the thyrotropic hormone. These two glands are evidently very closely related and balance each other. A deficiency in the thyroid stimulates the pituitary while an increase in thyroid secretion causes a reduction of pituitary activity. The anatomic changes in goitre must therefore be dependent upon the maintainance of a balance between the secretion of the thyroid and the thyrotropic hormone of the anterior pituitary.

From this mechanism with its associated anatomical changes the two principle clinical diseases associated with disturbed thyroid function may be listed as: (1) Hyperplastic goitre and (2) Colloid goitre. The hyperplastic or active goitre may be considered the developmental phase while the colloid or involutional goitre may be considered the recovery phase. secondary phases between these two principle ones are great in number depending upon the number of times the cycle recurs. This cycle is the only apparent anatomical response of which the thyroid is capable and is not a response to any specific disease. Excepting the true adenomata, the recurrence of this cycle produces the nodules found in long-standing goitre and gives rise to the terms diffuse and nodose goitres. Further changes such as hemorrhage, liquefaction, calcification and other forms of degeneration may occur which are secondary to the formation of nodes. Functional disturbances of the thyroid gland can then be grouped into diffuse or nodose hyperplastic and diffuse or nodose colloid goitres with added descriptions of the secondary changes.

In the hyperplastic type where the progress is very rapid and the patient is subjected to operation early, no nodes are found. The longer the history of goitre with the recurrent cycle of hyperplasia and involution the greater the probability of nodular formation. This explains why in any large group of goitres the nodose type will be found in much greater numbers in the colloid goitre, while the diffuse type will be found in much greater numbers in the hyperplastic goitre. It will be found, too, that the very large goitres weighing 200 to 300 grams will be among the long-standing nodose colloid goitres while the diffuse colloid goitres seldom weigh over 50 to 75 grams.

In our series of 1028 goitres removed at operation there were 481 of the colloid type, only 126 of which were described as diffuse while 355 were described as nodose goitres. There were 522 of the hyperplastic type, 334 of which were described as diffuse while only 188 were described as nodose goitres. That is, the incidence of nodose goitre was twice as large in the colloid while only one-half as large in the hyperplastic goitres. Of the whole colloid group very few weighed over 75 grams among the

TABLE 1

TYPE	NUMBER	DURATION	SIZE
			grams
Colloid:			
Diffuse	126	6 months- 5 years	30-150
Nodose	355	3 years -25 years	50-350
Hyperplastic:			
Diffuse	334	3 months- 1 year	30-120
Nodose	188	3 months-20 years	50-300
Adenoma	8		
Carcinoma	5		
Thyroiditis	9		
Branchial cysts	3		
Total	1,028		

diffuse while many weighed over 200 grams, (a few as high as 350 grams) among the nodose goitres. Of the whole hyperplastic group there was comparatively little difference in the size of the diffuse and nodose goitres. Very few of the diffuse weighed under 40 grams while very few of the nodose weighed over 100 grams. The case histories of these patients indicate the duration of diffuse goitres usually in the terms of months while the duration of nodose goitres is always in the terms of years (some as high as 25 and 30). That is, increased size and duration of time go hand in hand with nodular formation in the colloid type where involution occurs and recurs, but are less frequently

found in the hyperplastic type where active progression of the process continues without involution.

Of the remaining 25 surgically removed specimens in this series of 1028 goitres, there were 5 carcinomata, 3 branchial cysts, 9 described as thyroiditis, and 8 described as true adenomata. Those described as true adenomata were only those in which solid cords of cells were found with no attempt at follicle formation; the term foetal adenoma has not been used at all.

SUMMARY

The anatomic changes found in thyroid dysfunction are the only constant morphological findings and are produced by a mechanism described as the "hyperplasia-involution cycle."

The mechanism of this cycle and its associated anatomical findings gives rise to the two principle clinical diseases associated with disturbed thyroid function; hyperplastic goitre and colloid goitre.

Nodules in the goitre described as "nodose" are formed in thyroid glands which have been the seat of a recurrent hyperplasia-involution cycle.

In a series of 1028 surgically removed specimens there were twice as many nodose as diffuse colloid goitres and only about one-half as many nodose as diffuse hyperplastic goitres.

The time element and size of these goitres seem to be in accordance with the widely accepted explanation of the thyroid cycle in goitre.

In 1028 surgically removed specimens only 8 were described as true thyroid adenomata.

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A PRACTICAL CLASSIFICATION OF LEUKEMIC AND RELATED CONDITIONS*

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Sternberg (1903) published a comprehensive analysis of the primary diseases of the lymphatic and hemopoetic tissues. He devoted 157 pages to a consideration of the various characteristics of these and closed with a scheme for classifying them according to the presence or absence of three essential features: (1) The type of cells, whether lymphoid or myeloid, participating in the hyperplasia; (2) Escape of these cells (Ausschwimmung) into the circulating blood, and (3) Local invasive tumor growth (Geschwülsten). Varying combinations of these features enabled him to differentiate between: Lymphatic leukemia, Myeloid leukemia, Pseudoleukemia, Lymphoid Myeloma, Myeloid Myeloma, Leuko-sarcoma (Chloroleuko-sarcoma), Chloro-myelo-sarcoma, and Lympho-sarcoma.

Pathologists in general seem not to have sensed the significance and advantages of such a classification. At least the text books and monographs on pathology, with one notable exception, have failed to promulgate Sternberg's differentiation. MacCallum is the exception referred to. He stated:

"It appears, then, that if we know accurately all the cellular types existent in the bone marrow and in the lymphoid tissue, which are the blood forming tissues concerned, and if we assume that each is capable of undergoing an independent hyperplasia, we should be able to construct a tabulation of all the possible diseases arising in this way. This has indeed been done, just

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Presented before the Seminar on Tumors, American Society of Clinical Pathologists, May 1927, and before the American Association of Pathologists and Bacteriologists, May 1938.

as it was possible for Rokitansky to foretell what types of malformation of the heart might occur on the basis of the embryological development of that organ, and then years later to meet with cases, hitherto unknown, which realized each member of his scheme. The possible existence of unknown tumors has been foretold in the same way on a histogenetic basis."

In each of the six editions of his text-book, MacCallum discussed this group of diseases in a clear and logical fashion based upon the same differential characteristics proposed by Sternberg. He introduced some modifications and re-arrangement into Sternberg's outline, but used the same differential criteria and nomenclature.

Workers in other fields of science have found it not only convenient but absolutely necessary to classify types according to the presence or absence of certain distinctive features. Otherwise orders, classes, genera and species could not be sub-divided logically. A similar method is equally applicable and clarifying when applied to the group of conditions under consideration here. For years I have used MacCallum's scheme for differentiating the primary diseases of the blood-forming tissues. This has been of especial advantage in teaching this subject matter to medical students. This scheme has afforded such high satisfaction both in the differential diagnosis and in didactic presentation, that I recommend heartily its more extended use.

The outlines used by Sternberg and by MacCallum have been simplified somewhat. More recent terms have been substituted for the older nomenclature in a few instances, but the distinguishing features for each item have been retained. It has been necessary to supply an additional division for hyperplasias of the recticulo-endothelial cells. This outline does not apply to the hyperplasias incident to acute and chronic infections such as "glandular fever," typhoid, tuberculosis, syphilis and others. In other words, one is dealing with the non-inflammatory hyperplasias of hemapoetic tissues—the same group of conditions as discussed by Sternberg and MacCallum.

It is necessary to determine three essential items. First is the type of cells of which there is hyperplasia. This will establish

three main groups: Lymphoid, Myeloid and Reticulo-endothelial (Retothelial), which are designated by the Roman numerals I, II and III respectively. The second essential item is the presence of abnormal numbers of those cells in the circulating blood—leukemia. For convenience let this item be designated by the letter A. The third item is the presence of local invasive neoplastic growth, consisting of the same type of cells, somewhere in the body. Let this item be designated by the letter B. A plus or minus sign before either of these symbols will indicate its presence or absence respectively. On this basis a differential classification may be arranged as follows:

I. LYMPHOID HYPERPLASIA

- +A -B Lymphoid leukosis (lymphoid leukemia) (acute or chronic)
- -A -B Aleukemic lymphadenosis (pseudo-leukemia)
- +A +B Leukosarcoma
- -A +B 1. Primary neoplasm of lymphoid tissue (lymphosarcoma)
 - 2. Primary neoplasm of bone marrow (lymphoid or plasma cell myeloma).

II. MYELOID HYPERPLASIA

- +A -B Myeloid leukosis (or leukemia) (acute or chronic)
- -A -B Aleukemic myelosis
- +A +B Leuko-myelosarcoma
- -A +B 1. Myeloid tumor not of bone marrow (myelosarcoma)
 - 2. Myeloid tumor of bone marrow (myeloid myeloma)

III. RETICULO-ENDOTHELIAL HYPERPLASIA

- +A -B Monocytic leukosis (leukemic retotheliosis)
- -A -B Aleukemic retotheliosis (giant lymph follicle hyperplasia?)
- +A +B Leuko-retothelioma (or leuko-retosarcoma)
- -A +B 1. Neoplasm of retothelial tissue (Retothelioma, retosarcoma; Hodgkin's disease?)
 - 2. Retothelial neoplasm of bone (Ewing's tumor).

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Cases of plasma-cell myeloma and occasional reports of plasmacell leukemia suggest that provision should be made perhaps for the classification of these. If such cases shall be verified and substantiated it will be a simple matter to extend this outline to include plasma cells among the cell types participating in these non-inflammatory hyperplasias.

The following objections may be raised to this classification: There are cases in which it is doubtful whether the condition is of infectious origin, or whether a primary hyperplasia. Not in every case is it possible to determine whether the hyperplasia consists of lymphoid, myelocytic or monocytic cells.

In some cases the presence of a localized neoplastic growth may not be recognized clinically. Such growth may only be revealed by post-mortem examination.

Occasionally more than one type of cell may participate in the hyperplastic proliferation, and may appear in the leukemic blood.

In regard to these and other objections let it be observed that they apply with equal force to any proposed classification. The differential diagnosis for example between infection and acute myeloid leukemia is sometimes impossible. That fact is a criticism not of the scheme of classification, but of the available means for differentiating infections from other types of hyperplasia. If the type of cells in leukemic blood, in hyperplastic lymph nodes or in any type of neoplasm cannot be recognized, it is manifestly impossible to make a precise diagnosis either of the type of leukosis or of the neoplasm.

Let it be observed further that a failure to classify correctly any of the conditions outlined will not usually react unfavorably upon the welfare of the patient. Since each of these conditions usually ends fatally regardless of treatment, the chief benefit derived from an accurate diagnosis is the academic satisfaction derived in making it. If the three essential items of information cannot be secured from such examinations as one can make, then one will be denied the satisfaction of classifying that case appropriately by any system of differentiation.

Ability to record concisely the characteristic features of a given case is an advantage of considerable value. This greatly facilitates filing the cases appropriately and grouping them for comparative studies. For example, a case of leukemic lymphadenosis may have leukemic phases. Such a condition would be designated as I: $\pm A$ -B. The occurrence of transient periods of leukemic blood associated with myeloid myeloma would be indicated in the record as II: $\pm A$ +B2.

Those instances in which two types of cells participate in hyperplastic proliferation can be described logically by the use of symbols. For example a condition of leukosis in which both lymphoid and myeloid cells are present excessively in the blood may be recorded symbolically as I + II: +A -B.

The relationship of Hodgkin's disease to this classification cannot be determined finally until agreement is reached concerning its essential features. The slightly increased number of large mononuclear cells in the blood is hardly sufficient to be regarded as leukemic. If the characteristic type of the primary cellular proliferation in Hodgkin's tumor shall be determined to be reticulo-endothelial, then Hodgkin's disease will be represented by the symbols III: -A + B.

SUMMARY

A differential morphologic classification is proposed for the leukemias and for hyperplasias and primary neoplasia of the hemopoetic tissues. This classification does not apply to hyperplasias of inflammatory or infectious origin.

Its application requires accurate data concerning: (1) the type of cells involved, (2) the presence or absence of leukemic blood, and (3) the presence or absence of local neoplastic growth.

The conditions mentioned can be classified logically and systematically provided the required data are available. In the absence of such data no accurate classification is possible by any system.

Recording symbolically the essential features of a given condition contributes to concise accuracy and discourages loose thinking.

This system of classification facilitates greatly the didactic presentation and discussion of leukemic and related conditions.

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EDITORIAL

BEJEL-YAWS

The more accurate knowledge of syphilis dates from 1530. Since that date, no single sign or symptom of this disease is more important, or indeed more essential, to the diagnosis of acquired syphilis than the initial lesion. World-wide study of syphilis for 406 years has enabled physicians to make certain dogmatic statements in regard to it because these centuries have shown that its symptomatology follows certain rules which, laboriously worked out, have earned respect as laws.

We know, for example, that the number of cases of human acquired syphilis which begin without an initial lesion is so small as to be negligible, indeed this is so exceptional as to make us wonder if it ever occurs. The ability to think and observe on the part of the infected gauges the correctness of the history of initial lesions. With "primitives" this is utterly useless. With the better class civilized human being it is of greatest value.

Let us take a few examples. Suppose a nervous young man of 22 should consult his physician in some such manner as this: "Doctor, I am afraid I have syphilis." "What makes you think so?" "Well, I've never transgressed but once in my life and now I've an eruption on me." "How long since this transgression?" "3 months next Tuesday night at 10.45." "When did you first notice a genital sore?" "I have not had a genital sore!"

Inspection shows no trace of a genital lesion, and you, the physician, then look at the eruption, confident that whatever it is, it is not syphilis—erythema multiforme, pityriasis versicolor, sycosis barbae, psoryasis, acne vulgaris or what have you? But not syphilis.

Or, a girl of 19 with pretty face and engaging manner is brought

^{*}Bejel: nonvenereal syphilis. E. H. Hudson, Arch. Dermat. & Syph., 33, 994–1011, June, 1936.

to your office by her mother who is worried about a sore on her daughter's lip which will not heal. "How long have you had that?" "It has been there about a month, Doctor."

Inspection shows that the lesion is sclerotic, not painful and that a lymph gland draining the area is enlarged.

"Mary, have you kissed any young man about three weeks before you first noticed that sore?" "Yes, Doctor I allowed Willie Van Meter to kiss me at Sarah Bernhard's party which was (after calculation) two months ago next Friday." We take a specimen of Mary's serum and look up Willie Van Meter, being rather certain the serum will be positive and that Willie will still have a mucous patch or so which will enable us to discover the source of Mary's tragedy.

The diagnoses here are as follows: To the lad of 22 who had only sinned once in his life and who remembered the time to the hour and minute, the lad who had gone through all those years of hell in the two months of thinking about the future, you could confidently say, "John, you have no syphilis. What you have, we doctors call syphilophobia. Go and sin no more!"

But what are we to say to poor Mary who responded to that urge as old as life itself to love and be loved? The woman's specialty is rearing a family. If she is a proper woman, this is the breath of life to her, the great adventure for which she lives, moves and has her being. The diagnosis in Mary's case is innocently acquired syphilis. Willie Van Meter has done her a great injustice. But she may be completely cured and may live to be loved and honored by a family of healthy sons and daughters. In both the above cases the chancre (initial lesion) was the sine qua non of diagnosis.

Now let us consider the cases of human syphilis which come without evident initial lesion.

(1) Colles' Law. A child that is affected with congenital syphilis, its *mother* showing *no signs* of the disease, *will not* infect its mother (1837).

(2) Profeta's Law. A non-syphilitic child born of syphilitic parents is immune.

Now the "Colles mother" may have syphilis, though we may not be able to demonstrate it by any tests known to medicine. So also the "Profeta child" may be a syphilitic, even though we may find all tests negative. These are the only types of human syphilis in which it is difficult or impossible to demonstrate initial lesions. The "Colles Mother" and the "Profeta child" may later show symptomatic syphilis. The whole symptomatic spectrum of acquired syphilis may be shown by a chancre-less congenital syphilitic—early (1 month) or late (10 years) eruptions, gangosa, tabes, and general paralysis. Such congenital taint may remain latent through a long life and the offspring may be healthy. The presence or absence of a chancre is therefore of paramount importance in the diagnosis of the type of syphilis. On this basis there are three types of syphilis.

A. Venereally acquired syphilis with the chancre always situated upon the genitals. (We do not consider acquired syphilis as seen in sexual perverts as within the scope of these remarks.)

- B. Innocently acquired syphilis in which the chancre is extragenital.
- C. Congenital (Profeta) or "Colles" (mother) syphilis in which there is no demonstrable chancre. If we can prove the initial lesion was absent, and the patient a child we have prima facie evidence the syphilis is congenital. It cannot be otherwise. Now it makes no difference up to 10 or 12 years what time the chancreless eruption comes on, it is none the less congenital, whatever the mother may show as to serology or symptoms.

CONCLUSIONS

1. Any people incapable of acquiring *genital* (venereal) syphilis already have acquired or congenital syphilis. Yaws may thus be proven to be syphilis in the human being.

2. Bejel is congenital syphilis for this reason: initial lesions do not occur. It is inconceivable that the initial lesion could

escape years of search for it.

3. Yaws and Bejel are identical etiologically although they may differ slightly because of the climatic differences (chiefly humidity) under which each develops.

4. Yaws, bejel and syphilis are synonymous terms.

C. S. BUTLER.

NEWS AND NOTICES

ASSEMBLY OF LABORATORY DIRECTORS AND SEROLOGISTS

Sponsored by the U. S. Public Health Service, there was a meeting October 20th and 21st in Hot Springs National Park, Arkansas, of about 250 medical laboratory directors, serologists, syphilographers and chemists. As announced by the sponsor, the purpose of the meeting was to improve and render more widely available serodiagnostic tests for syphilis.

During the morning session of the first day, the originators of the more widely used serodiagnostic tests explained the necessity for adherence to conventional technics. The afternoon session was taken up with reports of evaluation studies, sensitivity and specificity ratings, and the relative value of doubtful reports.

That evening several score of those attending the meeting were guests at the home of Dr. D. C. Lee about 5 miles from Hot Springs for a barbecued supper. Between 8 and 10 P.M. there were demonstrations of the Eagle, Hinton, Kahn, Kline, Kolmer, and New York State Board of Health methods of serodiagnostic tests by the originators, with Senior Surgeon J. F. Mahoney in charge of demonstrations.

The morning session of the second day was somewhat less serene than the preceding sessions. The general subject of the papers dealt with educational and training requirements for laboratory directors and technicians. It was brought out that at present the directors of 22 state laboratories are not graduates in Medicine. It is well known, of course, that many laboratories wholly or principally occupied with the diagnosis of disease have no medical supervision. Indeed, persons without knowledge or training in the fundamental or basic medical sciences, especially pathology, hematology, and bacteriology are assuming responsibilities to the public which violate both the spirit and letter of medical practice acts in many states.

The President of the American Chemical Society, in discussing this subject, held that chemists had the same right to directorship of diagnostic laboratories as graduates in medicine, and that the society which he represents proposed to insist on and establish this right. He advanced the concept that the serodiagnosis of syphilis was nothing more nor less than colloidal chemistry. When it was brought to his attention that the directorship of most diagnostic laboratories implied a far wider and more diversified knowledge than the essential knowledge of biological chemistry, he admitted that such subjects, for example, as pathological anatomy, surgical pathology, and hematology were quite beyond the province of chemistry.

Following a long and somewhat acrimonious discussion of the subject of this

Saturday morning session, it became evident that neither the resolutions proposed by the committee in charge of this session, nor those of the chemists would prevail. A compromise resolution was adopted in which it was requested that the Surgeon General of the Public Health Service select a committee of one representative each from a number of medical and chemical organizations to give further study to the question of educational requirements.

At the final session Saturday afternoon two papers were presented on the subject of licensing or approval of laboratories for the serodiagnosis of syphilis by State Departments of Health. Although one of these papers dealt with this subject from the standpoint of the state director of laboratories, and the other from the standpoint of private enterprise, there was remarkable agreement between the two, not only in the matter of aims and purposes, but in the methods of accomplishing those purposes.

The resolutions offered by the Committee on Approving or Licensing were adopted with but one or two changes of minor character. Because of the influence which these resolutions may have in shaping the policies to be adopted by the U. S. Public Health Service and State Boards of Health, a copy is appended hereto.

In the writer's opinion, the assembly was fairly successful. It should harbinger a more comprehensive understanding of the laboratory problems which are common to both public health work and to the private or semiprivate practice of medicine. Neither one of these medical agencies is complete in itself. The position taken by representatives of the American Chemical Society, to speak frankly, was quite inauspicious and wholly selfish. It certainly contributed nothing to, indeed it detracted from the general purposes and objectives of the meeting. Until chemists and other nonmedical persons are able to assume some degree of legal responsibility to patients such as that required of the medical men and women, until their educational requirements and attainments are sanctioned by law, intending thereby to protect people from incompetency, they are quite unfitted as a group, from that standpoint if no other, to direct the activities of medical laboratories. A malpractice suit or two might serve to clarify this issue!

F. H. LAMB.

RESOLUTION OF COMMITTEE ON APPROVING FOR PERFORMANCE OF SERODIAGNOSTIC TESTS FOR SYPHILIS

The Committee is of the opinion that laboratory tests for syphilis should be performed on the same basis as are laboratory tests which aid in the control of other communicable diseases. Syphilis is a serious communicable disease and, as such, is a grave public health problem. The community has a profound obligation to protect itself against the spread of all communicable diseases through the application of proper public health measures.

States which have not already made provision for the approval of labora-

tories performing tests for syphilis, should as soon as possible arrange to approve laboratories which are under competent direction and meet adequate standards of efficiency.

Many communities now have insufficient laboratory facilities, including personnel, to handle the rapidly increasing number of tests required. Health departments providing laboratory services should arrange for these services to meet existing needs.

The following specific recommendations are made:

A. The Surgeon General of the United States Public Health Service be requested to establish in the Public Health Service a Department of Serology under a director, organized for fundamental research and for standardization of the reagents and procedures in the different serologic tests for syphilis, and for the evaluation of the performance of serodiagnostic tests for syphilis. Provisions should be made in the future for distributing standards as is done so successfully in the case of therapeutic sera.

B. The Surgeon General be requested to appoint a National Advisory Council on Serology of representative experts whose duties shall be purely advisory to the Department of Serology. A Council of eight members is suggested with terms expiring so that two appointments or reappointments are made each year. In addition, the Director of the Department of Serology should be a permanent member of the Committee. The Council may on request or on its own initiative express any opinion or submit recommendations as to policy or procedure that may serve to promote improvement of laboratory service in the diagnosis of syphilis in the United States.

C. A national census of all laboratories doing serologic work, including those doing spinal fluid and darkfield examinations, should be made at once by the United States Public Health Service.

D. Laboratory service in the serologic and other aids to the diagnosis of syphilis throughout the United States must be improved, extended and made more readily available:

1. By establishing standards for approval and approving laboratories that perform efficient tests. The director must be qualified for the position by training and special experience in the field and must assume responsibility for all of the examinations made in the laboratory and must determine if members of his staff are competent to perform the duties assigned to them.

2. All approved laboratories and all those who are seeking approval for the performance of serologic tests and the darkfield and spinal fluid examinations should be offered inspection of and conference with qualified representatives who have no other affiliation should be utilized except when a Special Consultant is requested by the State health authorities.

3. By the granting of subsidies, when necessary, to States to aid in the maintenance of properly qualified laboratories. The development of approval of local laboratory service is of primary importance. The federal grants-in-

aid should be used to assist in placing State laboratories on a satisfactory basis and to utilize and develop local laboratory facilities rather than to aid in the establishment or maintenance of branches of State laboratories which might hamper the development of local laboratories. All communities should have access to diagnostic laboratory service which will include those for the prompt examination of specimens of spinal fluid and for darkfield.

E. State and local appropriations should be sufficient to permit a laboratory to be conducted on a satisfactory basis including the performance of at least

two different tests when necessary.

F. When the United States Public Health Service is satisfied with the facilities offered by a State laboratory, approval but not necessarily licensure of local laboratories should be undertaken by the individual State. In cities to which State sanitary codes or similar legislation do not apply if the municipal laboratory has been found to be efficient, the municipality should undertake the supervision of its local laboratories. Otherwise the Public Health Service should coöperate with the State or large municipality in an effort to provide satisfactory facilities in such laboratories.

G. The Surgeon General should withhold federal grants-in-aid to State laboratories or the reallotment of federal funds to local laboratories, if such laboratories are performing inefficient tests for syphilis and are not attempting to further the development of reliable local laboratory service.

It is suggested that the Surgeon General, at his discretion may withhold all federal funds for venereal disease control from a State which fails in its laboratory obligations.

H. Standards for evaluation of the efficiency of laboratories.

Laboratories under competent direction and having adequate personnel and facilities for work, and whose directors have formally agreed in writing concerning the conduct of the work, will be considered as meeting at least minimum standards of efficiency in the performance of serologic tests for syphilis, provided:

1. The tests used are of sufficient scope to differentiate accurately sera of marked and slight specific activity in order to avoid prozone effects.

2. Positive reactions will not be obtained, or will be obtained in rare instances, with specimens from persons in good health apparently free from syphilis.

3. Definite reactions will be obtained in all but an extremely small percentage of instances with specimens from untreated patients with manifestations of secondary syphilis.

4. That the results reported by the laboratory will approximate the consensus of the results obtained by other approved laboratories with specimens from treated patients with syphilis.

5. A laboratory which does not meet these requirements should not be approved or, if approved, the approval should be withdrawn.

I. To aid in the evaluation of serodiagnostic tests for syphilis, those of spinal fluid, and darkfield examinations, periodic inspection of laboratories should be made. Approval should be renewable from year to year contingent on the maintenance of satisfactory work.

It is recognized that this report represents an exploratory expedition into a virgin and untrodden area and that the recommendations and suggestions should be acted upon with care and deliberation. It will be necessary to begin upon a small area and gradually increase the size and details of the venture.

I move the adoption of the report.

H. H. Hazen, Chairman. Frederick H. Lamb. John H. Stokes. N. A. Nelson. A. B. Wadsworth.

As illustrating what can be achieved by a Bureau of Social Hygiene in coöperation with the United States Public Health Service, the following list of laboratory services furnished by the City of New York without cost to the practicing physician is of interest: Darkfield for Spirocheta pallida; smears for gonococci and Ducrey bacilli; Wassermann tests; spinal fluid examinations for neurosyphilis; Frei tests for lymphogranuloma venereum; lumbar puncture; clinical consultation (diagnosis and treatment); neoarsphenamine and bismuth; epidemiologic service; Postgraduate instruction in the diagnosis and treatment of syphilis and gonorrhea; clinical conferences; listing of qualified physicians willing to treat referred patients at moderate fees.

Free services and facilities in connection with the Pneumonia Control Program include: free supplies of therapeutic and typing serum to hospitals; sputum typing and blood cultures by City laboratory; sputum containers; free typing serum to "commercial laboratories" willing to do free sputum typing; instruction in sputum typing; postgraduate instruction in serum therapy in pneumonia; consultation service of experts in pneumonia serum therapy.

As public health programs expand, it may be expected that similar facilities will be advocated in connection with programs developed for other diseases.

MISSISSIPPI VALLEY MEDICAL SOCIETY 1939 ESSAY AWARD

The Mississippi Valley Medical Society offers a cash prize of \$100.00, a gold medal and a certificate of award for the best unpublished essay on a subject of interest and practical value to the general practitioner of medicine. Entrants must be members of the American Medical Association. The winner will be invited to present his contribution before the next annual meeting of the Mississippi Valley Medical Society at Burlington, Iowa, September 27, 28, 29, 1939, the Society reserving the exclusive right to first publish the essay in its official publication—the Mississippi Valley Medical Journal (Incorporating the Radio-

logic Review). All contributions MUST NOT exceed 5000 words, be type-written in English in manuscript form, submitted in five copies, and must be received NOT later than May 1, 1939. Further details may be secured from

Harold Swanberg, M.D., Secretary, Mississippi Valley Medical Society, 209–224 W. C. U. Building, Quincy, Ill.

The 1938 winning essay, as well as several other essays which received meritorious consideration in the 1938 Essay Contest, appears in the Jan. 1939, issue of the *Mississippi Valley Medical Journal* (Quincy, Ill.)

Announcement for Medical and Scientific Publications for Immediate Release

The first American Congress devoted to a consideration of medical, nursing and other problems associated with human reproduction will be held in Cleveland, Ohio, from September 11 to 15, 1939, inclusive. It will be designated as The American Congress on Obstetrics and Gynecology. The promotion and sponsorship of The Congress has been delegated to the American Committee on Maternal Welfare, Inc. The latter includes the following organizations in its membership: American Association of Obstetricians, Gynecologists and Abdominal Surgeons, American College of Surgeons, American Gynecological Society, American Hospital Association, American Nurses Association, American Protestant Hospital Association, American Medical Association Section on Obstetrics and Gynecology, American Public Health Association, Central Association of Obstetricians and Gynecologists, Chicago Maternity Center, Maternity Center Association of New York, National Medical Association, National League of Nursing, National Organization for Public Health Nursing, New England Obstetrical and Gynecological Society, Pacific Coast Society of Obstetrics and Gynecology, Southern Medical Association, U. S. Bureau of the Census, U. S. Children's Bureau, U. S. Public Health Service.

The purpose of this Congress is to afford opportunities for discussing and publicising the problems associated with human reproduction and the health of women and new born babies. The value of more generally disseminated knowledge about the processes and problems of human reproduction and of the special diseases of the female generative organs and the new born is important in the maintenance of public health and therefore the interest of woman's welfare extends not only to the medical profession but to associated groups, including nurses, public health officials, hospital administrators, eugenists and many others.

Problems develop with the expansion of knowledge and these can be discussed most effectively at a meeting where many viewpoints can be intelligently discussed. Congresses, international and national, afford the means of presenting and discussing the advances in various fields of science and bringing

them to public attention. Obstetrics and gynecology in particular demand that wider association with allied groups, aside from the practitioners of medicine, which is so essential to the progress and welfare of the public. For these reasons the scope of the projected Congress has been extended beyond that of similar assemblies held in the past and will devote much attention to the wider public welfare aspects of problems which have been considered frequently of purely medical interest.

The last International Congress of Obstetrics and Gynecology was held in Amsterdam, Holland, in May of the present year. Its success stimulated a desire to hold a subsequent one in five years in another European country. It is felt that the difficulties associated with an international assembly, such as languages, expense, long distance travel, and limited participation, lack of common interest, call for a regional gathering in which opportunities for more general discussions would prevail. The proposed American Congress will therefore be modeled on different lines and include participation not only by medical groups but by those devoted, as already stated, to nursing, public health and institutional administration. The program will provide morning, afternoon and evening sessions, the details of which will be announced subsequently.

National, sectional and local specialist societies have approved the Congress and have made contributions for its support. It is desired that a wider representation be secured through the medium of contributing memberships, the cost of which has been placed at five dollars. Application may be made at the office of the Congress, 650 Rush Street, Chicago, Illinois. Early application is desirable and will serve as an indication of personal interest in the success of the undertaking.

In addition to the scientific sessions it is planned to provide for several evening meetings at which speakers of prominence will discuss the broader aspects of the subjects for the lay public. There will also be prepared a comprehensive exhibit—scientific, educational, technical and commercial, which should add greatly to the general interest of the Congress.

Cleveland is well adapted for a meeting of this kind. There are ample hotel facilities and the city is admirably located. The municipal auditorium, with suitable rooms for all types of meetings has been secured. Cleveland is a large railroad center and provides adequate transportation facilities, rendering access to the Congress easy from all parts of the United States and Canada.

The officers and committees thus far selected are as follows:

Executive committee:

Fred L. Adair, Chicago, Illinois, General Chairman, Robert D. Mussey, Rochester, Minnesota, Vice-Chairman, Sara B. Place, Chicago, Illinois, Secretary, Rudolph W. Holmes, Chicago, Illinois, Treasurer, Frederick H. Falls, Chicago, Illinois, Assistant Treasurer.

Program committee:

Frederick H. Falls, Chicago, Illinois, Chairman,

James R. McCord, Atlanta, Georgia, Secretary.

Medical Sub-Committee—James R. McCord, Atlanta, Georgia, Chairman.

Nursing Sub-Committee—Ruth Houlton, R.N., N.O.P.H.N., New York

Public Health Sub-Committee-Howard D. Mettel, Indianapolis.

Hospital Administration Subcommittee—Rev. A. Schwitalla, S.J.

Publicity and advertising committee:

Paul Gebhard, Chicago, Illinois, Secretary.

Medical Publicity Sub-Committee—George Kesmak, New York City, Chairman.

Promotion and arrangements committee:

Joseph L. Baer, Chicago, Illinois, Chairman.

Cleveland promotion and arrangements committee:

W. R. Barney, Cleveland, Chairman.

Educational and scientific exhibit committee:

Robert D. Mussey, Rochester, Minnesota, Chairman,

H. Close Hesseltine, Chicago, Illinois, Secretary.

Technical and commercial exhibit committee:

Bert W. Caldwell, Chicago, Illinois, Chairman,

Paul Gebhard, Chicago, Illinois, Secretary.

Budget and finance committee:

Walter T. Dannreuther, New York City, Chairman.

OBITUARY

Dr. Foy Clawson Payne was born at Hamilton, Ohio, in 1894. He died suddenly, of acute coronary heart disease, at Dayton, Ohio, on June 15, 1938. Doctor Payne was graduated from the Medical College of the University of Cincinnati in 1917. He served an interneship at the Jewish Hospital, Cincinnati, after which he was the Resident Physician in Pathology at the Cincinnati General Hospital. During the World War he served in this country and in France as a commissioned officer in the Medical Corps of the United States Army. Upon his return to Dayton after his discharge from the Army, he became associated with the late Doctor Ned Goodhue in the practice of clinical pathology. Doctor Payne succeeded Doctor Goodhue as Pathologist to the St. Elizabeth Hospital, of Dayton, in 1924. For several years, he was a Consultant in Pathology at the Veteran's Administration Facility at Dayton. At the time of his death, Doctor Payne was Pathologist to the St. Elizabeth Hospital and Good Samaritan Hospital, of Dayton, and the director of a large private laboratory.

Doctor Payne's interest in the field of clinical pathology began during his medical school days and continued uninterruptedly during his years of post-graduate training and during his service in the U. S. Army. He remained an enthusiastic student throughout his eminently successful career. He devoted a large share of his time and energies to the training of young physicians. He was a regular attendant at local, state and national medical society meetings. He was among the first of the clinical pathologists to be certified by the American Board of Pathology. Doctor Payne took great pride in his affiliation with the American Society of Clinical Pathologists.

An enthusiastic and capable clinical pathologist, a jovial and friendly medical colleague, a devoted husband and father, his sudden and untimely death, occurring at the full height of his career, came as a severe shock to a great host of friends.

BOOK REVIEWS

Handbook of Practical Bacteriology. By J. T. Mackie M.D., D.P.H., Professor of Bacteriology, University of Edinburgh, and J. E. McCartney, M.D., D.Sc., Director of Research and Pathological Services, London County Council. Cloth, Ed. 5, 586 pp. \$4.00. William Wood & Co., Baltimore, Maryland.

This is a familiar English text, intended primarily for the student, but of service also to the Laboratory worker in general.

Following an introductory section, Part II presents bacteriological technique and Part III a discussion of pathogenic microörganisms and bacteriological diagnosis. The present edition has been revised to include newer knowledge and modification. This is a useful book.

Handbook of Hematology. Edited by Hal Downey, University of Minnesota Medical School, Minneapolis, Minn. In four volumes, 3136 pp., 1148 illustrations including 50 colored plates. \$85.00. Paul B. Hoeber, Inc., Medical Book Department of Harper and Bros., New York.

In these long awaited volumes will be found perhaps the most comprehensive as well as the most authoritative discussion yet to appear in print of the broad subject of hematology.

Under the able editorship of Hal Downey thirty seven contributors, each of outstanding reputation in the field of hematology, discuss some special phase of the subject with the result that these volumes are encyclopedic in their comprehensiveness as shown by the chapter headings below:

Volume I: The Erythrocytes; The Polymorphonuclear Neutrophile Leukocyte; Eosinophile Leukocytes and Eosinophilia; The Mast Cells; Lymphocytes and Monocytes; Theories of Hematopoiesis; Functions of The Leukocytes; Blood Platelets and Megacaryocytes; Hemorrhagic Diathesis; Supravital Method of Studying Blood Cells; Application of The Supravital Method to the Study of Blood in Pathological Conditions; Evaluation of The Supravital Staining Method.

Volume II: Comparative Hematology; Embryology of Mammalian Blood; Normal Blood In Infants and Children; The Reticulo-Endothelial System; Monocytic Leukemia and Leukemic Reticulo-Endotheliosis; Fibroblasts and Histocytes; The System of Fixed Histocytes In The Liver; Lymphatic Tissue; Lymphatic Organs, Tissue Cultures of Blood and Blood-Forming Tissues.

Volume III: The Spleen; The Hemolymph Nodes; Normal Bone Marrow; Bone Marrow; Normal and Pathologic Physiology With Special Reference To

Diseases Involving The Cells of The Blood; The Myeloblast; Cytology of Pathologic Marrow Cells With Special Reference To Bone Marrow Biopsies; Myeloid Metaplasia; Classification of Anemias; Blood Pictures of Anemias and Anemias of Infancy and Childhood; Aplastic Anemia and Osteosclerosis; Pernicious Anemia; Chronic Hereditary Hemolytic Jaundice; Sickle-Cell Anemia; Ovalocytosis.

Volume IV: Polycythemia; The Pyrrol Pigments, With Particular Reference to Normal and Pathologic Hemoglobin Metabolism; Infectious Mononucleosis; Heterophilic Antibody Reaction In Infectious Mononucleosis; The Blood Pictures of The Infectious Diseases Occurring Primarily in Childhood; The Action of Benzol, Roentgen Rays and Radioactive Substances On The Blood and Blood-Forming Tissues; Agranulocytosis and Granulocytopenia; Leukocytosis, Leukemia; Leukemia In Infants and Children; Lymphosarcoma and Leucosarcoma; Index.

It has long been appreciated that an understanding of hematology in all its varied aspects is impossible without a previous understanding of the fundamentals upon which it is based. These basic aspects are fully covered and thoroughly discussed.

Moreover, in many of its phases hematology is a controversial subject and a particularly valuable aspect of this work is the full expression of divergent opinions on subjects open to discussion. The Editor has wisely allowed his contributors full freedom of thought and in so doing has given the reader a well rounded survey of hematological problems in all their aspects.

Valuable, also, are the extensive bibliographies following each chapter.

The illustrations are not only numerous but excellently chosen and beautifully reproduced. All in all the work represents a triumph of the printer's art without which no medical nor working reference library can be considered complete. For this will be, without doubt a standard and authoritative reference text for years to come.

While obviously of value to the hematologist, physiologist, research worker and pathologist, it is equally useful to the clinician, whether specialized or physician at large. In fact, there is no field of medical endeavor in which these volumes will not prove useful.

Insulin, Its Chemistry and Physiology. By Hans F. Jensen, Ph.D., Associate,
 Laboratory for Endocrine Research, The Johns Hopkins University. Cloth.
 252 pp. \$2.00. The Commonwealth Fund, New York.

In this monograph the author presents a comprehensive review of the developments in the studies of the chemistry and physiology of insulin. The volume is authoritative and covers the literature to January 1938. It can be recommended as a valuable survey.

The Sex Criminal. By Bertram Pollens. With a Foreword by Richard A. McGee, Warden, Riker's Island Penitentiary, New York City. Cloth. 211 pp. 5 illustrations. \$2.00. The Macaulay Co., New York.

In this book Dr. Pollens, who is senior psychologist to the Penitentiary of The City of New York, presents the problem of the sex criminal from the standpoint of the psychologist, illustrating his thesis from his experience with such individuals who have fallen into the hands of the law.

His conclusion is that usually arrived at: that the solution of this problem—if solution there be—lies in a new and broader educational program.

Biography of the Unborn. By Margaret Shea Gilbert. Cloth. 132 pp. 36 figures. \$1.75. The Williams & Wilkins Co., Baltimore, Md.

This book is an attempt to present for the lay reader the story of human development from the union of ovum and spermatozoon until the moment of birth. The story is well and clearly told in an interesting fashion and the book may well take its place among the better texts having this as their theme.

Diseases of The Thyroid, Parathyroid and Thymus. By André Crotti, M.D., F.A.C.S., LL.D., R.I.C.S. Ed. 3. Half-leather. 1229 pp. 262 illustrations and 39 colored plates. \$20.00. Lea and Febiger. Philadelphia.

This is a third enlarged and thoroughly revised edition of an outstanding work and represents, perhaps, one of the most comprehensive—if not the most comprehensive—texts on this subject. Dr. Crotti has had a vast experience in this field amply reflected in his book. He has not hesitated to express fully the opinions based upon his experience. His book is a stimulating contribution without which a reference library cannot be complete.

The Etiology of Trachoma. By Louis A. Juliannelle, Ph.D., Chairman of The Trachoma Committee, Washington University, St. Louis. Cloth. 248 pp. 10 plates. The Commonwealth Fund, New York.

This volume, based upon several years' intensive study, presents a critical and analytical review of the present concepts of the etiology of trachoma.

Those interested in this subject will find the volume authoritative and comprehensive. An extensive bibliography is appended.

Big Fleas Have Little Fleas, Or Who's Who Among The Protozoa. By ROBERT HEGNER, Professor of Protozoology in the School of Hygiene and Public Health, The Johns Hopkins University. Based on Messenger Lectures, Cornell University, 1937. Cloth. 285 pp. 126 figures. \$3.00. The Williams & Wilkins Co., Baltimore, Md.

Lewis Carroll would not only have enjoyed this book but gnashed his teeth to think he had not written it.

Those who have regarded protozoology as a difficult and dry subject will receive this book with whoops of joy. For, without sacrificing accuracy and while maintaining a highly scientific standard, Professor Hegner has written an account of protozoa which is a joy to read.

Not only the book itself but its illustrations are sui generis. Witty, amusing and at times even hilarious, the story of protozoa as Professor Hegner tells it, is of absorbing interest.

This is a book that should not be missed. It is difficult to imagine anyone reading it without interest and profit as well as amusement, and impossible to imagine seeing it and not wanting to own it.

CONCERNING THE METHOD PROPOSED FOR RE-PORTING THE SEROLOGICAL REACTIONS FOR SYPHILIS AS POSITIVE, DOUBTFUL AND NEGA-TIVE*

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For almost thirty years the results of Wassermann tests have been reported as strongly positive, moderately positive, weakly postive, doubtful and negative; later the same terminology was adopted in relation to some of the various flocculation tests for syphilis so that the terms have become widely known in the serology of syphilis.

Recently, however, the Committee on the Evaluation of Serodiagnostic Tests for Syphilis of the United States Public Health Service, cooperating with the American Society of Clinical Pathologists, has advocated reporting the results of all serum tests for syphilis as "positive," "doubtful" and "negative." Under this plan all definitely positive reactions hitherto recorded as strongly or moderately positive are reported merely as "positive" while weaker reactions are reported as "doubtful."

It is well known that when the complement-fixation or any of the flocculation tests are conducted with a single dose of serum that a certain minimum amount of syphilis reagin will yield a ++++ or strongly positive reaction and that amounts of reagin over and above this minimum cannot give a stronger one. For this reason tests employing one or two and even three different amounts of serum cannot give a quantitative reaction in the sense of measuring the amount of reagin present and I prefer calling them "qualitative reactions." However, when less than the minimum amount of reagin is present in the single

^{*}Received for publication November 8, 1938.

dose of serum employed, the reactions are weaker ranging from +++ to +. Under these conditions a strongly positive or ++++ reaction implies that the single dose of serum employed contained a sufficiently large amount of reagin to give such a result but without measuring beyond this point while a + + +, ++ or + reaction indicates that less than this amount of reagin was present; in this sense the results are at least partially quantitative. If, for example, 4 units of reagin give a + + + + reaction, 100 units cannot give a stronger one and the serum of a patient starting off with 100 units and reduced to 10 units under treatment will still give a ++++ reaction and possibly mislead the physician into the conclusion that treatment has produced no serological improvement. For this reason I advocated a quantitative complement-fixation test about sixteen years ago employing five different amounts of serum to secure a more accurate quantitative reaction with the hope that the largest dose would detect the presence of small amounts of reagin and the fifth dose exhaust the reagin in the great majority of sera.

With this in mind the first dose was 0.1 c.c. (now changed to 0.2 c.c.) ending with 0.0025 c.c. as the fifth or last dose (now changed to 0.005 c.c.) in an effort to secure a workable range for routine practical use although realizing that a larger range of serum doses would be still better. Furthermore, it was apparent that the conduct of tests with varying amounts of serum would give some safeguard against error due to prezone reactions where an occasional serum may give a weaker or even a falsely negative reaction with a large dose of serum and a true positive reaction with a smaller amount.

I believe, therefore, that a quantitative complement-fixation test employing at least five amounts of serum or cerebrospinal fluid is to be preferred and especially when the tests are conducted in relation to the treatment of syphilis. For diagnostic purposes, however, two or three doses suffice in a complement-fixation test as the difference between the percentages of positive reactions with quantitative and qualitative tests by my method is practically negligible.

But with both, and especially the latter, I believe that reac-

tions should be reported as strongly positive, weakly positive, doubtful and negative rather than as positive, doubtful and negative as advocated by the Cooperative Committee. Under "strongly positive" I propose including those hitherto regarded as strongly or moderately positive (++++ and +++ reactions) since these almost invariably indicate the presence of syphilis when the tests are properly conducted and malaria, leprosy and infectious mononucleosis are excluded; under "weakly positive" I propose including those hitherto reported as ++ or +, as the great majority indicate the presence of syphilis under similar conditions while \pm reactions are reported as "doubtful." In other words, I advise splitting the "positive" report of the Cooperative Committee into "strongly positive" and "weakly positive."

My reasons are entirely clinical. In the first place it must be realized that the great majority of serum tests for syphilis are conducted for physicians who have not had the opportunity for acquiring a special knowledge and skill in the detection of the disease. Under the conditions they must depend upon serological diagnosis to a far greater degree than is true of expert syphilologists. Given a patient with chronic latent syphilis and especially one with a negative history and in good general health, a serological report of "positive" may be discarded as being of no significance since syphilis was not suspected or evidences of it detected clinically. But if the report is "strongly positive," as is not infrequently the case, such disposal of it is not likely to occur. It may be stated, however, that a report of "weakly positive" may be likewise discarded under these circumstances but hardly more so in my experience than one reported merely as "positive."

In the second place, if syphilis is suspected clinically and the serum reactions are reported as "strongly positive" or even "weakly positive" it has been my experience that more practicing physicians will sit up and take notice than when receiving a report of only "positive."

In the third place, a patient starting off before treatment with a "strongly positive" reaction who later shows a "weakly positive" reaction gives the physician at least some evidence of serological improvement under treatment, whereas a mere "positive" report under such circumstances leaves him and the patient without this encouragement.

I am among those who think it is generally inadvisable to reveal to patients the results of their serological tests under treatment because they may place entirely too much emphasis upon their value and significance but yet in practice I have found it frequently necessary to reveal the results and, furthermore, I have found this advisable and necessary in many instances in order to secure proper cooperation in treatment as I am among those who believe that persistently positive reactions are indicative of persistent infection requiring therapeutic management.

However, one point in favor of reporting positive reactions merely as "positive" is the fact that there is not infrequently a marked discrepancy between the degrees of positiveness and the clinical status of the patient. For example, a man barely able to walk with tabes dorsalis may give only a "weakly positive" reaction whereas another in excellent general health and without detectable clinical evidences of the disease may give a "strongly positive" reaction. I have long sought to explain this marked discrepancy in clinical and serological status on the basis that the degree of positiveness depends upon the amount of reagin in the blood and that this in turn depends upon the degree of spirochetal infection but that clinical manifestations in chronic syphilis depend not so much upon the numbers of spirochetes present in the tissues as upon their location. In other words, that the signs and symptoms of chronic syphilis will be greater in relation to the physiological importance of the tissue or organ infected so that a few spirochetes in the posterior columns and posterior roots of the spinal cord may produce infinitely more damage with but little reagin in the blood than a large number of spirochetes tucked away in the skeletal system with a large amount of reagin in the blood.

Furthermore and very importunately, there is no absolute correlation between the degree of positiveness of complementfixation and the various flocculation tests for syphilis. In those laboratories doing both the reactions may be strongly positive with one and weakly positive with the other, which makes it almost as difficult for the laboratory to offer an interpretation to the clinician as when one is positive and the other negative. This is an additional argument in favor of the plan of the Cooperative Committee for reporting positive reactions just "positive." Certainly the latter is to be welcomed by serologists although in all truth the serologist feels a grateful release in responsibility by being able to report the degree of positiveness with the tests employed and leaving interpretation entirely up to the clinician.

TABLE 1
Comparative Kolmer and Kahn (Standard) Reactions with 1395 Sera

	TOTAL	KAHN STANDARD REACTIONS					
XOLMER REACTIONS		Mod. to strongly pos.	Weakly pos.	Doubtful	Negative		
W-14-1-41		per cent	per cent	per cent	per cent		
Moderately to strongly posi- tive	1,048	60061.2	26.6	8.0	4.2		
Weakly positive	255	12.5	10 39.6	27.1	20.8		
Doubtful	92	4.4	30.4	2 40.2	25.0		

For example, as shown in table 1, of 1048 syphilitic sera giving moderately to strongly positive Kolmer complement-fixation reactions in my laboratory,* 61.2 per cent gave moderately to strongly positive Kahn (standard) reactions. Of 255 giving weakly positive Kolmer reactions, 39.6 per cent gave weakly positive Kahn reactions; of 92 giving doubtfully positive Kolmer reactions, 40.2 per cent gave doubtfully positive Kahn reactions. In other words, the interpretation of the two reactions agreed in 39.6 to 61.2 per cent.

Table 2 shows the results observed with the same sera in the Kline (diagnostic) reaction. Of 1048 giving moderately to strongly positive Kolmer reactions, 55.3 per cent gave similar Kline reactions; of 255 giving weakly positive Kolmer reactions,

^{*}I am indebted to Mrs. Elsa R. Lynch for the compilation.

30.6 per cent gave weakly positive Kline reactions while of 92 giving doubtful Kolmer reactions, 66.3 per cent gave doubtful Kline reactions.

More correlation, however, was shown (table 3) with a group of 8293 sera tested in the Pennsylvania State Laboratories.† Of 6603 giving moderately to strongly positive Kolmer reactions, 73.6 per cent gave similar Kline (diagnostic) reactions; of 1236

TABLE 2

Comparative Kolmer and Kline (Diagnostic) Reactions with 1395 Sera

		KLINE DIAGNOSTIC REACTIONS				
KOLMER REACTIONS	TOTAL	Mod. to strongly pos.	Weakly pos.	Doubtful	Negative	
		per cent	per cent	per cent	per cent	
Moderately to strongly posi-						
tive	1,048	55.3	26.5	15.7	2.4	
Weakly positive	255	10.2	30.6	45.9	13.3	
Doubtful	92	2.1	19.5	66.3	12.1	

TABLE 3

Comparative Kolmer and Kline (Diagnostic) Reactions with 8293 Sera (Pennsylvania State Dept. of Health Laboratories)

	TOTAL TESTED	KLINE DIAGNOSTIC REACTIONS		
KOLMER REACTIONS		Mod. to strongly pos.	Weakly pos.	Doubtful
		per cent	per cent	per cent
Moderately to strongly positive	6,603	73.6	22.1	4.3
Weakly positive	1,236	37.5	43.5	18.9
Doubtful	454	23.1	44.9	31.9

giving weakly positive Kolmer reactions, 43.5 per cent gave weakly positive Kline reactions and of 454 giving doubtful Kolmer reactions, 31.9 per cent gave doubtfully positive Kline reactions. The percentage of agreement between the interpre-

†I am greatly indebted to Dr. Louis Tuft, Director of the Pennsylvania State Laboratories, for permission to use these data; also to Miss Carola E. Richter of his staff for their compilation.

tation of the two reactions varied therefore from 31.9 to 73.6 per cent.

As shown in tables 1, 2 and 3 however, the degree of positivity between the Kahn (standard) and Kline (diagnostic) reactions varied only from 0.1 to 26.1 per cent whereas the Kolmer reactions differed from the Kahn (standard) in from 28.8 to 60.4 per cent and from the Kline (diagnostic) in from 26.4 to 68.1 per cent.

But as shown in table 4, the differences are not nearly as great when the percentages include negative reactions. This table summarizes the results of 10733 sera examined in my laboratory during the past year. Insofar as moderately to strongly positive reactions are concerned it will be noted that only 4.7 per

TABLE 4
COMPARATIVE KAHN, KLINE AND KOLMER REACTIONS WITH 10733 SERA

REACTIONS	KAHN (STANDARD)	(DIAGNOSTIC)	KOLMER
	per cent	per cent	per cent
Mod. to strongly pos	6.5	5.9	11.2
Weakly positive		3.3	2.4
Doubtful		6.9	2.7
Negative	87.8	83.9	83.7

cent variation occurred between the Kahn (standard) and Kolmer reactions with 5.3 per cent variation between the Kline (diagnostic) and Kolmer reactions. As between the Kahn and Kline reactions the difference was only 0.6 per cent.

With weakly positive reactions the difference between the Kahn (standard) and Kolmer reactions was only 0.5 per cent and between the Kline (diagnostic) and Kolmer reactions only 0.9 per cent. The difference between the Kahn and Kline reactions was only 0.4 per cent.

With doubtfully positive reactions the difference between the Kahn (standard) and Kolmer reactions was only 0.1 per cent and between the Kline (diagnostic) and Kolmer reactions 4.2 per cent. Between the Kahn and Kline reactions the difference was 4.1 per cent.

With negative reactions the difference between the Kahn (standard) and Kolmer reactions was only 4.1 per cent and between the Kline (diagnostic) and Kolmer reactions only 0.2 per cent. Between the Kahn and Kline reactions the difference was 3.9 per cent.

I believe that the same reagin or antibody is responsible for positive complement-fixation and flocculation reactions but because of factors at present unknown and beyond control or adjustment it is to be expected that in routine work positive Kahn (standard) reactions will vary from positive Kolmer reactions (including doubtfully positive reactions in both) in from 0.1 to 4.7 per cent of sera insofar as the degree of positivity is concerned and that the variation between Kline (diagnostic) and Kolmer reactions varies from 0.9 to 5.3 per cent. In view of this close agreement I believe that reactions with all three methods may be justifiably reported as "strongly positive" (to include moderately to strongly positive reactions), "weakly positive," "doubtful" and "negative."

On the other hand, however, the differences may be properly interpreted as an additional reason for reporting all positive serological reactions merely as "positive" as advocated by the Cooperative Committee on the basis that reporting one as "strongly positive" by one method and "weakly positive" or "doubtful" by another may be just as confusing to the average physician as reporting one as "positive" and the other as "doubtful" or "negative."

But it has not worked out this way in my clinical experience. Rather when one was "strongly positive" and the other "weakly positive" or "doubtful" the physician has been much more careful in interpretation than when both were reported only as "positive." It is true that many physicians have been just as much confused as when one was reported "positive" and the other "negative" but certainly not more so.

Therefore, I am in favor of and advise a compromise between the older method and the newer or recent method for reporting the results of serological reactions in syphilis. My proposal is to group moderately to strongly positive reactions by both complement-fixation and flocculation tests as "strongly positive" since the results of serologic surveys conducted by the Cooperative Committee have shown that these almost invariably indicate definite serologic evidences of syphilis. Reactions formerly reported as "weakly positive" are reported as such as likewise those giving "doubtful" and "negative" reactions. In other words, I merely propose splitting the "positive" reaction of the Cooperative Committee into "strongly positive" and "weakly positive" for reasons previously discussed.

CONCLUSIONS

For reasons set forth in this paper it is proposed to report the results of complement-fixation and flocculation reactions in syphilis as "strongly positive," "weakly positive," "doubtful" and "negative" instead of as "positive," "doubtful" and "negative" as advocated by the Cooperative Committee of the U. S. Public Health Service and the American Society of Clinical Pathologists.

OCCURRENCE OF A NON-SPECIFIC SUBSTANCE IN GUINEA PIG SERUM FIXED BY ANTIGEN IN THE WASSERMANN TEST*

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In our experience with the Kolmer⁵ complement fixation test, we have been impressed at infrequent intervals by the occurrence of false positive reactions and the zone phenomenon in an entire series. These reactions were made particularly conspicuous in spinal fluid examinations, since all other findings were normal, such as the colloidal gold curve, the protein content, cell count, and flocculation tests.

Personal inquiry of other well-informed serologists revealed similar experiences. The fact that we participated in the second serologic conference conducted by the United States Public Health Service⁷ without the incidence of false positive reactions, and attained a sensitivity of 71.4 per cent as compared to 59 per cent, that of the author control, is convincing evidence that we were dealing with a sporadic factor. Nevertheless, the problem was of such serious import that it demanded prompt solution, particularly since we place greater reliance on the Kolmer complement fixation test than on flocculation tests.

Recently, this disconcerting problem presented itself, when an entire series of Wassermann tests, with few exceptions, showed inhibition of hemolysis in the first two tubes. All serum controls were completely hemolyzed as was the hemolytic system control. The antigen control, however, showed complete inhibition. Titration of the amboceptor and complement units, the latter in the presence of antigen, indicated no diminution in titre.

Our first assumption was that the antigen was involved in this

^{*} Received for publication May 26, 1938.

phenomenon. In order to determine whether it was the factor influencing our reaction, we repeated the anticomplementary titration, using both normal serum and saline. According to routine procedure, the pooled complement which had been used the previous day was reactivated by the addition of fresh serum obtained from a healthy male guinea pig. The highest dilution antigen tested (1:40) showed complete inhibition of hemolysis in both titrations, one employing normal serum and the other saline. Identical results were obtained in the retitration of a different antigen prepared according to the same formula six months previously. Duplicate titrations using fresh pooled complement showed no inhibition of hemolysis in dilutions as low as 1:4. These findings indicated that complement was a factor in the inhibition, and the antigens per se were not involved.

Since the complement titration in the presence of antigen gave no warning of non-specific reactions likely to occur in the Wassermann test, we believed that some factor other than the well known protective action of serum was to be considered. Eagle¹ emphasizes the spontaneous deterioration of complement during prolonged ice box incubation and states that every Wassermann series should include a control for the deterioration of complement under the conditions of the test. Therefore, two series of tests were made to determine the deterioration factor of complement. In no instance was there any inhibition of hemolysis in the tubes containing two full units of complement, the amount used in our Wassermann test, even after incubation in the re-There was marked deterioration, however, in tubes containing less than two full units. Since no inhibition occurred in the complement titration, incubated at 37°C., it became obvious that the inhibitory effect was due to the reaction between complement and antigen at 8°C.

Again, we encountered another series in which all spinal fluid tests were strongly positive, serum tests exhibited the zone phenomenon, the antigen control was inhibited, and the hemolytic system control clear. Kline³ tests and colloidal gold reactions on five of the six spinal fluids were negative. Many of the sera were negative when tested by the Kline and Mazzini⁶

tests. The degree of inhibition was greater in the sera showing weakly positive flocculation tests than in those which were negative. Since the new antigen*8 had been recently tested for anticomplementary activity, it was apparent that one or more component parts of the fresh, pooled complement must react with the antigen after incubation at 8°C. producing inhibition of hemolysis. Therefore, the following preliminary test was instituted to study the hemolytic activity of each guinea pig serum at 8°C.:

To 1.1 c.c. isotonic salt solution was added 0.1 c.c. normal serum, inactivated at 56°C. for fifteen minutes, 20 units of antigen, and 0.3 c.c. 1:30 dilution of complement. After four hours incubation at 8°C., two units of amboceptor and 0.5 c.c. of 2 per cent suspension of sheep cells were added. The tubes were shaken well and placed in the water bath at 37°C. for ten minutes. A second test was performed on each complement, substituting 0.125 c.c. normal spinal fluid for 0.1 c.c. serum. Only guinea pig sera which produced complete hemolysis within ten minutes were considered satisfactory. This procedure was followed for several weeks during which time over forty guinea pigs were tested. All sera produced complete hemolysis in the preliminary test, and the antigen and hemolytic system controls of the test proper showed prompt hemolysis on the subsequent day.

Several weeks later, an interesting phenomenon was observed after the addition of amboceptor and sheep cells to the preliminary test of the three new guinea pig sera labeled A, B, and C. A and B complement produced hemolysis within ten minutes while C complement showed complete inhibition after incubation for one hour. The pooled complement, consisting of approximately equal volumes of serum from pigs A, B, and C exhibited no unusual reaction in the amboceptor or complement titration at 37°C. At last the solution of the problem appeared at hand,

^{*}A primary ether extraction was made prior to acetone extraction in the Alternative method. The purpose of this procedure, recommended by Dr. Yagle, was "to remove adventitious substances which might give non-specific reactions." This antigen will be referred to as #3.

namely that occasionally one encounters guinea pig sera which contain a substance that combines with antigen at 8°C. producing false positive reactions.

The following morning after addition of the hemolytic system to the Wassermann series in which ABC complement had been employed, the results which we anticipated soon were apparent. After incubation for one hour, the antigen control was completely inhibited, hemolytic system control clear, and most bloods were positive in tubes 1 and 2 with a marked zone reaction apparent in several tubes. All serum controls but two were clear.

TABLE 1

	COM	APLEMENT	TITRATION*	WASSERMANN TEST (KOLMER)				
	37°C.	Ambo- ceptor unit	8°C.	Blood	Cerebro- spinal fluid	Antigen control	Hemolytic system control	
Complement	1:75	1:12,000	75% inhibi- tion in all tubes	False posi- tives. Zone phenom- enon	False posi- tives	Complete in- hibition	Hemolyzed	
A + B complement Equal volumes of complement (A +	1:50	1:12,000	1:50	No false positives	No false positives	Hemolyzed	Hemolysed	
B) and C complement	1:50	1:12,000	75% inhibi- tion in all tubes	False positives. Zone phenomenon	False posi- tives	Complete in- hibition	Hemolyzed	

^{*} Two full units.

Obviously, the inhibitory effect of C serum in the pooled complement was believed to be the cause of the non-specific reactions in this series. To prove this contention, the pigs A, B, and C used on the previous day, were again bled. Serum A and B were combined and serum C kept separate. Duplicate titrations were set up with A + B complement and with C complement. (See table 1.) One series was incubated at 37° C. and the other series placed in a refrigerator for three hours at 8° C. before the addition of the hemolytic system. As anticipated, the titre of A + B complement, incubated at 8° C. was the same

as that of the 37° C. titration, whereas the ice box incubation series of C complement showed 75 per cent inhibition in all tubes. It is of particular interest that the titre of C complement incubated at 37° C., was higher than that of A + B complement, incubated under the same conditions. (See table 1.) Blood and cerebrospinal fluid tests employing A + B complement and

TABLE 2
RELATION OF C SERUM TO VARIOUS ANTIGENS

	ALTER- NATIVE ANTIGEN	ALTERNA- TIVE ANTI- GEN (OLD)	ANTIGEN NO. 3	NON-CHOLES- TERINIZED ANTIGEN	G. C. ANTIGEN	KLINE	MAZZINI
Complement fix- ation tests	100 per cent inhibition	100 per cent inhibition	100 percent		No inhi- bition		
Flocculation tests						Negative	Negative

TABLE 3
PRELIMINARY TEST AT 8°CENTIGRADE

,	SALINE	ANTIGEN	C COMPLEMENT		DEGREE OF IN- HIBITION OF HEMOLYSIS
	cc.	ce.			per cent
0.1 cc. normal inactivated serum	1.1	0.5	0.3 cc. 1:30 dilution	Shake tubes gently	75
0.2 cc. normal inactivated serum	1.0	0.5	0.3 cc. 1:30 dilution	and place in the	25
0.5 cc. normal cerebrospinal fluid	0.7	0.5	0.3 ec. 1:30 dilution	refrigerator at 8°C.	100
0.125 cc. normal cerebrospinal fluid.	1.1	0.5	0.3 ec. 1:30 dilution	for three hours	100
0.1 cc. saline	1.1	0.5	0.3 ec. 1:30 dilution	Add hemolytic sys-	100
0.125 cc. normal cerebrospinal fluid.	1.6	None	0.3 cc. 1:30 dilution	tem and incubate for ten minutes at 37°C.	None

Occurrence of a non-specific substance in guinea pig serum which is fixed by antigen in the Wassermann test.

all of our stock antigens compared favorably with the Kline and Mazzini tests; the antigen control and hemolytic system control were completely hemolyzed. Another series employing ABC complement and the older antigen gave the same inhibiting effect as that observed in the series in which antigen No. 3 had been used. The antigen control was inhibited and the hemolytic system control was clear.

It is evident from table 2 that C serum contains a thermostable substance which combines with both cholesterinized and non-cholesterinized antigen at 8°C., and fixes the available complement. The serum control indicates no anticomplementary reaction. Flocculation tests with Kline and Mazzini antigens are negative. Therefore, this reacting substance is different from that reported by other workers.² It is inherently present in the animal observed, and is not influenced by diet. Genetic experiments are now under way to determine the inheritance factor.

SUMMARY AND CONCLUSIONS

A study has been made to explain the cause of non-specific complement fixation in the Wassermann test. Evidence is presented which indicates that the serum of some guinea pigs contains a substance which fixes complement in the presence of antigen at refrigerator temperature. It is heat stable and does not react at 37°C. Since this substance will not be detected in the daily titrations of amboceptor and complement, it is suggested that each guinea pig serum employed as complement be subjected to the described preliminary test.

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GUINEA PIG SERUM IN RELATION TO PREZONE AND NON-SPECIFIC WASSERMANN REACTIONS*

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Occasionally, and especially during the warm summer months, the Kolmer quantitative complement-fixation test for syphilis yields prezone reactions like -244 or -1344 with non-syphilitic human sera which are very disturbing because when they occur the phenomenon affects practically all of the tests being conducted, requiring their repetition. In most instances the first two tubes carrying 0.2 and 0.1 c.c. of serum show complete or almost complete hemolysis due to the protective action of serum, so that prezone reactions are much less likely to occur in qualitative tests employing these amounts. With syphilitic sera the reactions are usually 44444 regardless of whether they contain small or large amounts of syphilis reagin.

These prezone reactions also occur with normal spinal fluids and in the absence of the protective action of serum, may yield reactions like 22222 or 44444. Sometimes in a set of tests the reactions with negative sera are ---- while those with negative spinal fluids give prezone reactions.

Under these circumstances the serum controls on all sera and spinal fluids usually show complete hemolysis as likewise the hemolytic system control, whereas the antigen control shows inhibition of hemolysis. As a general rule the complement employed gives the usual exact unit of 0.3 or 0.35 c.c. of 1:30 dilution with the usual unit of hemolysin although, not infrequently, the complement is below average in hemolytic activity

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giving an exact unit like 0.4 or 0.45 c.c. of 1:30 with a lower unit of hemolysin. Inexperienced technicians usually suspect that these prezone reactions are due to defective hemolysin but since this reagent is remarkably stable it is never found responsible; invariably the difficulty is due to the complement and the occurrence of such prezone reactions may easily lead inexperienced serologists into the very regrettable error of reporting non-specific or falsely positive reactions.

The phenomenon may occur more frequently with the new C.L. antigen re-enforced with acetone insoluble lipoids¹ than with plain C.L. antigen, probably because of its higher sensitivity, although since the adoption of the method of Boerner and Lukens²· ³· ⁴, for determining the optimum dose of antigen to employ the antigen factor in these prezone reactions has been materially reduced.

It has been our impression that these prezone reactions are due to the use of complement unduly sensitive to the occult anticomplementary effects of antigen even though the latter, in the usual dose of 0.5 c.c. of about 1:600, is at least 80 to 100 times less than its anticomplementary unit as determined by titration (usually not higher than 0.5 c.c. of 1:6). It is especially likely to occur with the complement sera of sick pigs, those below normal weight and those which have been previously used for titration of antitoxin, inoculation for tubercle bacilli, etc. Another possible responsible factor is deterioration of complement during the primary incubation of 15 to 18 hours at 6 to 8°C. as suggested by Eagle⁵, although we have found that the unit of complement titrated in the presence of antigen with a primary incubation of one hour in a water bath at 37°C. is usually the same as in titrations employing a primary incubation of 4 hours or even 15 to 18 hours at 6 to 8°C. At all events, the phenomenon is in important relation to the amount of complement employed in the sense that prezone reactions occurring with a 1:30 dilution of complement usually do not occur when a 1:20 dilution is employed and suggesting that the unusual and undue susceptibility of the complement of some pigs in high dilution to the inactivating influence of antigen is largely responsible.

Recently, however, Giordano and Carlson⁶,* have presented evidence indicating that these non-specific and prezone reactions may be due to the fact that the complement sera of some guinea pigs contains a substance which fixes complement in the presence of antigen at refrigerator temperature, which is heat stable and does not react at 37°C.

Of course it is well known that a large percentage of the heated sera of normal rabbits, dogs, mules, cattle and sheep are capable of fixing complement with syphilis and particularly with various bacterial antigens⁷, and as recently shown by Greene and his colleagues⁸ the Kahn, Kline, Ide, Eagle and Laughlen flocculation tests also give a surprisingly large number of positive reactions with the normal sera of the dog, rabbit, goat, horse, sheep, chicken and cow.

As stated elsewhere, the cause or mechanism of these non-specific reactions with the sera of the lower animals is unknown. It is worthy of note that fresh unheated sera are much less likely to give them. Heating at 55–56°C. greatly increases their incidence while heating at 62°C. greatly reduces them. For this reason I have thought that the phenomenon was due to some obscure colloidal reaction involving the physical chemistry of animal sera profoundly increased by heat and on this basis advise heating rabbit sera at 62°C. instead of 55–56°C. for thirty minutes before using them for Wassermann or other complement-fixation tests.

It is well known that unheated guinea pig complement sera may contain tuberculosis complement fixing antibody capable of yielding falsely positive complement-fixation reactions with human sera, but until Giordano and Carlson reported their observations there was no suspicion that the fresh unheated complement serum of some guinea pigs may contain a substance capable of fixing complement with syphilis antigen in view of the fact that the Wassermann test has an extremely high practical specificity when properly conducted and when such diseases as malaria, leprosy and infectious mononucleosis are excluded.

^{*} I am greatly indebted to Dr. Giordano and Miss Carlson for kindly furnishing me with a copy of their paper prior to its publication.

However, in view of the possibility of guinea pig complement sera containing a substance capable of fixing complement with syphilis antigen Kolmer¹⁰ adopted the method of titrating complement in the presence of antigen in the technic of his complement-fixation test for syphilis as employed by Thompson¹¹ on the basis that a primary incubation of complement and antigen for one hour in a water bath at 37°C, would allow for fixation by any natural antibody or non-specific substance in the complement and reflect this effect by increasing the unit. However, Giordano and Carlson state that incubation at 37°C. is insufficient for this purpose but our results herein summarized have shown that it occurs in an incubation of one hour in a water bath at 37°C. although not usually as frequently or in as high degree as at 8°C. for 4 hours as they advise. The matter of practical importance therefore is whether or not it is advisable to titrate complement in the presence of antigen with a primary incubation of 4 or even 15 to 18 hours at 6° to 8°C, as a means for adjusting the unit and dose of complement more nearly under the conditions of the Kolmer complement-fixation test.

NON-SPECIFIC WASSERMANN REACTIONS WITH UNHEATED GUINEA PIG SERA

Giordano and Carlson advise testing the serum of each individual guinea pig as follows and using for complement only those sera giving complete hemolysis or negative reactions:

(a) Dilute 0.1 c.c. of normal non-syphilitic human serum with 1.1 c.c. of saline solution and heat at 56°C. for 15 minutes. Of course the serum should be free of anticomplementary effects.

- (b) Or normal spinal fluid may be used in dose of 0.125 c.c. unheated (preferred).
 - (c) Add dose of antigen and 0.3 c.c. of 1:30 dilution of complement.

(d) Incubate at 8°C. for 4 hours.

(e) Add 2 units of hemolysin and 0.5 c.c. of 2 per cent suspension of sheep corpuscles.

(f) Mix and incubate in a water bath at 37°C. for 10 minutes when the readings are made. Only those complement sera giving complete hemolysis are regarded as satisfactory for use in complement-fixation tests.

We have tested each of the complement sera of 74 large, healthy guinea pigs with both normal human serum and spinal fluid as likewise without either serum or spinal fluid, employing C.L. antigen re-enforced with acetone insoluble lipoids¹ and making the readings at the end of ten minutes' secondary incubation in a

water bath at 37°C. as well as at the end of an hour. The results are summarized in table 1.

As shown in this table, 63.5 per cent of the 74 complement sera gave positive reactions in the presence of heated normal human serum after a primary incubation of 4 hours at 8°C. followed by a secondary incubation in a water bath at 37°C. for 10 minutes as advised by Giordano and Carlson; 36.5 per cent gave positive reactions in tests conducted with normal spinal fluid at the same time and in the same manner. When the reactions were recorded at the end of one hour secondary incubation those conducted with serum gave 9.5 per cent positive reactions and 9.4 per cent with spinal fluid. It seems to us therefore that the secondary incubation should be one hour instead of ten minutes.

TABLE 1
RESULTS OF TESTS WITH THE COMPLEMENT SERA OF 74 HEALTHY GUINEA PIGS

	BECOND-	TESTS WITH NEG. SERUM			TESTS WITH NEG. 8P. FLUID			PLAIN TESTS*					
PRIMARY INCUBATION	ARY INCUBA- TION (WATER BATH)	Neg.	Weakly pos.	Strongly pos. ‡	Per cent pos.	Neg.	Weakly pos.	Strongly pos.	Per cent pos.	Neg.	Weakly pos.	Strongly pos.	Per cent pos.
4 hrs. refrig.§	10 min. 1 hr.	27 67	19 6	28 1	63.5 9.5	47 67	16 3	11 4	36.5 9.4		12 1	4 3	21.3 5.4
$1 \text{ hr.water} \begin{cases} \text{bath} \end{cases}$	10 min. 1 hr.	9 59	29 8	36 7	87.8 2.0	56 70	14 3	4	24.3 5.4		19 3	4 2	31.1 6.7

^{*} With no serum or spinal fluid.

In tests conducted without either serum or spinal fluid, 21.3 per cent gave positive reactions at the end of a secondary incubation of 10 minutes and 6.7 per cent at the end of one hour, showing that complement in the small dose of 0.3 c.c. of 1:30 dilution may undergo some fixation in the presence of antigen at 8°C. for 4 hours even though, as previously stated, this may not increase the unit of complement.

In tests conducted with a primary incubation of one hour in a water bath at 37°C. and read at the end of ten minutes secondary incubation, 87.7 per cent gave positive reactions with normal serum, 24.3 per cent with normal spinal fluid and 31.1 per cent with neither. When read at the end of one hour secondary incubation, positive reactions were observed with only 2 per cent in tests conducted with normal serum, 5.4 per cent with normal spinal fluid and 6.7 per cent with neither.

t + and ++.

¹⁺⁺⁺ and ++++.

[§] At 8°C.

These results have shown therefore that the fresh unheated complement of the sera of some normal healthy guinea pigs may undergo complete or partial "fixation" or inactivation by syphilis antigen at 8°C, for 4 hours in confirmation of the results reported by Giordano and Carlson. They state, however, that this does not occur at 37°C. but according to our results this occurs in equal or even greater degree. Furthermore, as previously stated, we believe that 10 minutes secondary incubation in a water bath at 37°C. is entirely too brief and that the readings are better made at the end of an hour. On this basis about 10 per cent of guinea pigs would have to be excluded as a source of complement if the tests are conducted with a primary incubation of 4 hours at 8°C. and from 2 to 6 per cent if the tests are conducted with a primary incubation of one hour in a water bath at 37°C. Of course the percentage of positive reactions is likely to vary in different colonies of guinea pigs and other investigators may find somewhat higher or lower figures, but it is apparent that if the tests are conducted with a primary incubation of 4 hours at 8°C. and a secondary incubation of 10 minutes that a very large percentage of pigs would be found unsuitable.

We are unable to state why Giordano and Carlson have advised including normal human serum or spinal fluid in conducting the tests except to state that they regard either as necessary and representing a true control on the exact conditions of the test (12). If positive reactions are due either to the presence of a natural complement-fixing substance in guinea pig complement or to its inactivation by antigen during the primary incubation, it would appear unnecessary to use normal human serum or spinal fluid in conducting the tests. As shown in table 1, if the readings are made at the end of one hour secondary incubation, 5.4 to 6.7 per cent of guinea pig sera show incomplete hemolysis due to "fixation" or the inactivating influence of antigen in tests without human serum or spinal fluid in both primary incubations as compared with 2 to 9.5 per cent in tests conducted with normal human serum or spinal fluid. Under the conditions it appears that normal human serum or spinal fluid and especially the former, may have a protective influence and that the Kolmer method of titrating complement in the presence of antigen without normal human serum or spinal fluid with a primary incubation of one hour in a water bath at 37°C. is sufficient although a primary incubation of 4 hours at 8°C, or even 15 to 18 hours at the same temperature as employed in the conduct of his complement fixation test for syphilis may be better and is being investigated at the present

Whether or not the positive reactions are due to the presence of a natural substance or antibody in guinea pig complement capable of fixing complement with syphilis antigen, to fixation (inactivation) of complement by antigen alone, deterioration of complement or to a combination of these factors during the primary incubation will be shortly discussed, but there can be no doubt that Giordano and Carlson are correct in their assertion that such guinea pig complement sera are apparently responsible for prezone reactions.

As shown in table 2, Kolmer quantitative complement-fixation tests were conducted with 10 normal non-syphilitic sera employing for complement the serum of a guinea pig giving a ++++ reaction in the Giordano-Carlson preliminary test employing a primary incubation of 4 hours at 8°C. and read at the end of 10 minutes' secondary incubation. All gave prezone reactions. A duplicate set of tests was conducted with a mixture of complement sera from five pigs, three of which gave negative and two strongly positive (++++) reactions in the preliminary tests. All of these gave prezone reactions although

TABLE 2
Kolmer Complement-Fixation Tests with 10 Normal Human Sera

SERA	SINGLE COMPLEMENT*	MIXTURE OF COMPLEMENTS
3	- 2 4 4 4	1 2 2
4	4 4 4	1 1 1
6	- 1 4 4 4	1 3 3
7	3 4 4	1 1
9	4 4 4	1 3 3
14	- 4 4 4 4	2 4 4
15	4 4 4	1 2 2
17	- 2 4 4 4	1 3 3
18	- 2 4 4 4	1 3 3
19	- 1 4 4 4	1 2 2

* This complement gave a ++++ reaction in the preliminary test with normal serum employing a refrigerator primary incubation of 4 hours at 8°C. and read after a secondary incubation of 10 minutes in a water bath. Unit was 0.4 cc. of 1:30; unit of hemolysin 0.5 cc. of 1:4000. Antigen control ++++; hemolytic system control: complete hemolysis.

† Mixture of 5 complement sera; 3 gave negative and 2 strongly positive reactions (+++) in preliminary tests with normal serum employing a refrigerator primary incubation of 4 hours and read after a secondary incubation of 10 minutes in a water bath. The mixture included the serum of the pig furnishing the single complement (++++). Unit 0.35 cc. of 1:30; unit of hemolysin 0.5 cc. of 1:6000. Antigen control +; hemolytic system control: complete hemolysis.

not in as high degree as observed in the tests with the single ++++ complement. The unit of complement of the single animal was 0.4 c.c. of 1:30 and the hemolysin unit 0.5 c.c. of 1:4000; the unit of complement with the mixture was 0.35 c.c. of 1:30 with a hemolysin unit of 0.5 c.c. of 1:6000.

Twenty-seven additional tests with normal non-syphilitic sera were conducted with the single complement serum of a pig showing a ++ reaction in the preliminary test. The unit of this complement was 0.35 c.c. of 1:30 with a hemolysin unit of 0.5 c.c. of 1:6000. All of the tests gave prezone reactions as shown in table 3.

Duplicate tests were conducted with a mixture of complement sera from 7

pigs, 3 of which gave negative, 2 weakly positive (+ or ++) and 2 moderately positive (+++) reactions in the preliminary tests. The unit of complement of the mixture was 0.35 c.c. of 1:30 and the unit of hemolysin 0.5 c.c. of 1:6000,

TABLE 3
KOLMER COMPLEMENT-FIXATION TESTS WITH 27 NORMAL HUMAN SERA

SERA	BINGLE COMPLEMENT*	MIXTURE OF COMPLEMENTS
1	1 3 4	
2	3 4	
3	2 4	
4	2 4 4	
5	4 4	
6	1 4	
7	3 4 4	
8	2 4 4	
9	2 2 4	
10	3 4 4	
11	2 3	
12	1 2 3	
14	1 4 4	
15	4 4	
18	3 4 4	
20	1 3 4	
21	1 4	
22	1 3	
23	1 3	
24	1 3 3	
25	1 3 4	
26	1 3 4	
28	4 4	
32	1 4 4	
34	1 3 4	
35	4 4	
37	3 4	

^{*} This complement gave a ++ reaction in the preliminary test with normal serum employing a refrigerator primary incubation of 4 hours at 8°C. and read after a secondary incubation of 10 minutes in a water bath. Unit was 0.35 cc. of 1:30; unit of hemolysin 0.5 cc. of 1:6000. Antigen control ++; hemolytic system control: complete hemolysis.

[†] Mixture of 7 complement sera; 3 gave negative, 2 weakly positive (+ or ++) and 2 moderately positive (+++) reactions in preliminary tests with normal sera employing a refrigerator primary incubation of 4 hours at 8°C. and read after a secondary incubation of 10 minutes in a water bath. Unit 0.35 cc. of 1:30; unit of hemolysin 0.5 cc. of 1:6000. Antigen and hemolytic system controls: complete hemolysis.

both being the same as with the single complement. As shown in table 3, none of these tests gave prezone reactions, probably because the inclusion of 4 complements giving weakly to moderately positive preliminary reactions were counterbalanced by the 3 giving negative reactions.

NON-SPECIFIC WASSERMANN REACTIONS WITH HEATED GUINEA PIG SERA

Of special interest is whether or not prezone reactions are due to a substance in guinea pig sera capable of fixing complement with syphilis antigen, to inactivation or "fixation" of complement by antigen alone, to deterioration of complement or a combination of these during the primary incubation in the conduct of complement-fixation tests for syphilis. Curiously enough it appears that those investigators, including Kolmer, who have studied the sera of normal rabbits, dogs, mules and other of the lower animals for their capacity for yielding non-specific positive complement-fixation and flocculation tests for syphilis^{7, 8} have not included the sera of guinea pigs, probably because there was no suspicion of unheated or fresh guinea pig complement sera carrying a natural complement fixing substance in view of the very high practical specificity of the serologic tests for syphilis under proper technical conditions.

The results summarized in table 1 are those we have observed with fresh unheated guinea pig sera employing re-enforced C.L. syphilis antigen.

The sera of 132 additional pigs were heated in a water bath at 55°C. for 15 to 20 minutes and tested by the qualitative Kolmer complement-fixation test for syphilis in dose of 0.1 c.c. with re-enforced C.L. antigen and with the regular primary incubation of 15 to 18 hours at 6 to 8°C. followed by ten minutes in a water bath at 37°C. before the addition of two units of hemolysin, 0.5 c.c. of 2 per cent sheep corpuscle suspension and a secondary incubation of one hour at 37°C. when the results were read. A duplicate test with each serum was conducted at the same time and in the same manner except that the primary incubation was one hour at 37°C. in a water bath.

As shown in tables 4 and 5, forty-three or 32.6 per cent gave positive reactions in tests employing a primary incubation of 15 to 18 hours at 6 to 8°C. with a secondary incubation of one hour in a water bath, while 25 or 18.9 per cent gave positive reactions in tests employing a primary incubation of one hour in a water bath. Practically all of these reactions were only weakly positive but the results have shown that the *heated* sera of some normal guinea pigs may contain a natural complement fixing substance or antibody for syphilis antigen or that heating guinea pig sera confers upon some of them the capacity for fixing complement as has been found true of rabbit, dog and mule sera. As shown in table 4, the inclusion of the sera of positive reacting pigs in groups of 3 to 7 sera for pooled complement did not appear to affect the hemolytic unit of pooled complement or the unit of hemolysin. Furthermore, in the conduct of quantitative Kolmer complement fixation tests with the pooled complements including the sera of positively reacting pigs, the reactions were completely satisfactory in 22 of the 25 sets of tests indicating that there were no prezone reactions with

completely hemolysed serum, antigen and hemolytic system controls while "fair" with the remaining three sets, meaning that the antigen and some of the serum controls were not completely hemolysed although with complete hemolysis of the hemolytic system controls.

TABLE 4
Kolmer Complement-Fixation Tests with Heated Guinea Pig Serum

SET NO.	NUMBER PIGS USED	NO. GIVING POS. BEACTIONS REFRIG. INCUB.*	NO. GIVING POS. REACTIONS WATER BATH INCUB. †	UNIT OF COMPLE- MENT 1:30	UNIT OF HEMOLYSIN	RESULTS OF KOLMEI
1	6	1	1	0.3	1:8000	Satisfactory
2	5	0	0	0.35	1:6000	Satisfactory
3	6	2	1	0.4	1:5000	Satisfactory
4	6	2	2	0.3	1:8000	Satisfactory
5	5	2	1	0.3	1:2000	Fair
6	6	2	1	0.3	1:6000	Satisfactory
7	5	1	1	0.3	1:6000	Satisfactory
8	6	0	0	0.25	1:10000	Satisfactory
9	3	0	0	0.35	1:4000	Satisfactory
10	4	0	0	0.25	1:6000	Satisfactory
11	5	1	0	0.25	1:8000	Satisfactory
12	5	2	2	0.3	1:5000	Satisfactory
13	5	3	1	0.35	1:5000	Fair
14	5	4	2	0.3	1:5000	Satisfactory
15	5	0	0	0.3	1:6000	Satisfactory
16	5	3	0	0.35	1:6000	Satisfactory
17	5	3	3	0.35	1:4000	Satisfactory
18	5	2	1	0.3	1:5000	Satisfactory
19	6	3	1	0.3	1:6000	Fair
20	5	4	2	0.35	1:8000	Satisfactory
21	5	0	0	0.35	1:5000	Satisfactory
22	7	3	3	0.35	1:5000	Satisfactory
23	6	3	1	0.3	1:6000	Satisfactory
24	5	1	1	0.3	1:6000	Satisfactory
25	6	1	1	0.25	1:12000	Satisfactory

^{* 15} to 18 hours at 6 to 8°C. followed by 10 minutes in a water bath.

Kahn standard tests were also conducted with the sera of 88 of these pigs and, as shown in table 5, 9.1 per cent gave positive reactions but all gave negative Kline diagnostic reactions with the sera of the same 88 pigs, probably because of the much shorter period of contact between serum and antigen at room temperature.

In other words, as shown in table 1, about 9.5 per cent of unheated guinea

[†] Water bath at 37-38°C. for 2 hours.

pig sera gave positive reactions after a primary incubation of 4 hours at 8°C. whereas, as shown in table 5, about 32.6 per cent of heated (55°C. for 15 to 20 minutes) gave positive reactions with a primary incubation of 15 to 18 hours using a secondary incubation of one hour in a water bath in both series. With a primary incubation of one hour in a water bath (table 1), 2 per cent gave reactions with unheated and 18.9 per cent with heated sera (table 5), the

TABLE 5
KOLMER, KAHN AND KLINE TESTS WITH HEATED GUINEA PIG SERA

KOLMER QUANT	TTATIVE TESTS*	KAHN STANDARD	KLINE DIAGNOSTIC
Refrigerator;	Water bath§	TESTS	TESTS
32.6% pos.	18.9% pos.	9.1% pos.	All neg.

* Conducted with sera of 132 pigs.

† Conducted with sera of 88 pigs.

‡ 15 to 18 hours at 6 to 8°C. followed by 10 minutes in water bath; secondary incubation 1 hour water bath.

§ 2 hours at 37-38°C. in water bath; secondary incubation 1 hour water bath.

TABLE 6
THE INFLUENCE OF HEAT UPON NON-SPECIFIC WASSERMANN REACTIONS BY
GUINEA PIG SERUM

SERA NO.	REACTIONS WITH UNHEATED SERUM	REACTIONS WITH SERUM HEATED AT 56°C. FOR 15 MIN.	REACTIONS WITH SERUM HEATED AT 62°C FOR 30 MIN.
1	_	++	+
2	_	_	_
3	-	-	_
4	_	+++	++
5	-	_	_
6	_	-	_
7	-	_	_
8	_	+	_
9	_	_	_
10	_	_	_

secondary incubation being likewise one hour in a water bath in both series. These results indicate, therefore, that heating guinea pig sera at 55°C. materially increases their capacity for yielding non-specific Wassermann reactions, as has been found true of the normal sera of rabbits, dogs, mules and other domestic animals as previously stated.

This influence of heat is also reflected in the results of Kolmer qualitative complement-fixation tests conducted with the sera of 10 guinea pigs employing

fresh unheated sera and after these had been heated at 55°C. for 15 to 20 minutes and at 62°C. for thirty minutes in dose of 0.1 c.c.

As shown in table 6, all of the unheated sera gave negative reactions, which may have been due in part to the fact that they furnished larger amounts of complement; after heating at 55°C. for 15 to 20 minutes three of the sera gave positive reactions whereas after heating at 62°C. for thirty minutes these were reduced and indicating that the substance or factor in guinea pig serum as the result of heating at 55°C. is reduced or removed by heating at 62°C. and exactly similar in these respects to the influence of heating at 62°C. in the case of normal rabbit sera.

DISCUSSION

As previously stated, prezone and non-specific complement-fixation tests are due to factors involving the complement employed and especially in the Wassermann test; it is my impression that they are much less frequently encountered in bacterial complement-fixation tests.

That the fresh active complement sera of approximately 2 to 10 per cent of normal guinea pigs may contain a non-specific substance capable of fixing complement with syphilis antigen with a primary incubation of 4 hours at 8° or one hour at 37°C. and employing a secondary incubation of one hour in a water bath at 37°C. appears to be established, the percentage of positive reactions being much higher when the secondary incubation is reduced to a period of ten minutes as employed by Giordano and Carlson.

Furthermore, it is entirely likely that the use of such complement may be at least partly responsible for prezone and non-specific complement fixation reactions. Deterioration of complement during the primary incubation appears to be of much lesser importance, but I am still of the opinion that the use of complement unusually sensitive to the inactivating influence of human serum, spinal fluid and antigen is likewise of importance in the production of prezone and non-specific reactions without involving the presence of a non-specific complement fixing substance. As previously stated, it has been my experience that the prezone reactions are especially likely to occur during the hot months and with the sera of ill-nourished, underweight and previously inoculated pigs irrespective of season.

Unquestionably the kind of antigen and especially the dose employed is also a factor of importance, involving as it does not only chemical factors, but the serum-antigen proportions so importantly influencing the colloidal reactions involved.

That falsely positive or prezone reactions may be erroneously reported as positive reactions by inexperienced technicians in the serologic tests for syphilis cannot be denied and is greatly to be regretted. Fortunately, the chances of this error are very much less in the case of experienced serologists.

When the pooled sera of three or more guinea pigs are used for complement the chances of falsely positive prezone reactions are very slight in the Kolmer complement-fixation test for syphilis where the pooled complement is titrated in the presence of antigen with a primary incubation of one hour in a water bath at 37°C. At least the high practical specificity of the test has been amply proven when malaria, leprosy and infectious mononucleosis have been excluded. For example, in the first serologic survey conducted in 1934 under the auspices of the U.S. Public Health Service in cooperation with the American Society of Clinical Pathologists, the specificity rating of tests conducted by Kolmer was 100 per cent with a sensitivity rating of 75.913. In the survey of 1936 the specificity rating was likewise 100 per cent with a sensitivity rate of 78.214. In the last survey of 1938 the specificity rating was again 100 per cent with a sensitivity rate of 88.2 per cent.

Heating guinea pig sera at 55 to 56°C. for 15 to 20 minutes does not remove the non-specific complement fixing substance; on the contrary it appears to increase it while heating at 62°C. for 30 minutes reduces it. In these respects guinea pig sera behave in the same manner as the sera of normal rabbits, dogs and other domestic animals and to the best of my knowledge this fact has been first elicited by the results of the investigation reported in this communication.

SUMMARY

1. Prezone or non-specific complement-fixation tests for syphilis are apparently due to the presence of a non-specific substance sometimes present in guinea pig complement serum

(Giordano and Carlson), to the fact that the sera of some guinea pigs are unusually sensitive to the inactivating influence of antigen and serum or spinal fluid or to a combination of these factors.

2. The complement sera of 36.5 to 63.5 per cent of 74 guinea pigs gave positive complement fixation reactions with syphilis antigen and normal human serum or spinal fluid after a primary incubation of 4 hours at 8°C. followed by a secondary incubation of 10 minutes at 37°C. but the percentage was reduced to about 9.5 per cent when the secondary incubation was prolonged to the usual period of one hour.

3. In tests conducted without the presence of normal human serum or spinal fluid the percentage of positive reactions was 21.3 with a secondary incubation of 10 minutes which was reduced to 5.4 with a secondary incubation of one hour.

- 4. In tests conducted with normal human serum or spinal fluid and a primary incubation of one hour at 37°C., the percentage of positive reactions was from 24.3 to 87.8 employing a secondary incubation of 10 minutes and from 2 to 5.4 with a secondary incubation of one hour. In tests conducted without either normal human serum or spinal fluid the percentage of positive reactions was 31.1 with a secondary incubation of 10 minutes which was reduced to 6.7 with a secondary incubation of one hour.
- 5. About 32.6 per cent of the *heated* sera of 132 guinea pigs gave positive Kolmer complement-fixation tests conducted with a primary incubation of 15 to 18 hours at 6 to 8°C.; with a primary incubation of one hour in a water bath 18.9 per cent gave positive reactions. About 9.1 per cent of 88 of these animals gave positive Kahn reactions (standard test) but all gave negative reactions with the Kline (diagnostic) test.
- 6. Heating guinea pig serum at 66 to 56°C. for 15 to 20 minutes increased the percentage of positive reactions whereas heating at 62° for 30 minutes reduced them; in these respects guinea pig sera behave in the same manner as the sera of normal rabbits, dogs and other domestic animals but not to the same extent as the sera of normal rabbits.
 - 7. The method of titrating pooled complement of three or more

healthy fully nourished guinea pigs in the presence of antigen with a primary incubation of one hour in a water bath at 37°C. as employed in the Kolmer complement-fixation test greatly reduces the chances of prezone or non-specific reactions so that in the hands of experienced serologists the chances of reporting such as positive reactions are reduced to a minimum as shown by the high practical specificity of the test under proper technical conditions when malaria, leprosy and infectious mononucleosis were excluded.

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THE LAUGHLEN TEST FOR SYPHILIS*

Comparison with 1000 Kline, 1100 Kolmer and 100 Kahn Tests

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There are two main varieties of tests for syphilis in common use, complement-fixation tests and the precipitation or flocculation tests. Between these two varieties there is very little choice as, in good hands, both have been found to have about the same degree of accuracy. In both types of test the antigens are tissue extracts, most of them derived from non-syphilitic tissues, varying widely in their mode of preparation, but not greatly in quality. In both types of tests the non-specific tissue extracts (antigens) are presumed to unite with the specific syphilitic antibodies (specific reacting substance) but the exact nature of the reaction is not known. In these non-specific tests it is quite probable that we are not testing for true anti-The reacting substance is probably in chemical structure a globulin or pseudo-globulin liberated in response to the presence of spirochetal products. With such biologically nonspecific tests it has been found impossible to produce universally accurate results, for many factors inherent in the patient's body, as well as in the technic of the tests, will be responsible for variations in the results. That a completely satisfactory test has not vet been devised is attested by the number of new ones appearing in the literature from time to time.

An *ideal* test for syphilis would be completely specific giving no false positive or false doubtful results in known non-syphilitic persons. Such a test has not yet been developed. A *satisfactory* test is required to give one per cent, or less, false positive

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results. The clinician must remember, therefore, that a false positive or doubtful result may be obtained on about one patient out of one hundred tested by any method. The first two American serologic conferences showed that only the Kolmer complement-fixation test and the Kahn and Kline flocculation tests (diagnostic) qualified under this requirement. All other methods, (with the possible exception of the Eagle and Hinton flocculation tests), will give more than one per cent false positive results.

An *ideal* test for syphilis should also be so sensitive as always to detect syphilis when it is present. Such a test has not yet been devised as, when an attempt is made to increase the sensitivity of any test for syphilis beyond a certain point, specificity is lessened and more false positive results occur. To the clini-

cian, specificity is more important than sensitivity.

In the third evaluation of sero-diagnostic tests for syphilis¹ from figures obtained in the laboratories of the originators of the tests, the Kolmer complement-fixation test was shown to have a sensitivity of 88.2 per cent; the Kline 83.9 per cent; the Kahn 91.8 per cent. In other words, these three methods will fail to detect on an average of from 8.2 to 16.1 per cent of syphilis in a known syphilitic population of treated and untreated cases, most of the undetected cases being in the groups of early and treated cases. In the secondary cases these three methods have a sensitivity of 100 per cent; in the untreated cases the sensitivity ranges from 85 to 90 per ccent.

Occasionally, a blood specimen will give a negative result with one method and a positive result with another. This signifies merely that the patient has only a small quantity of specific reacting substance in the blood and that one test is a little more

sensitive than the other in that particular case.

One of the chief difficulties with flocculation or precipitation methods has been the difficulty of reading the reactions and this, no doubt, is in great part responsible for the variations in results of these tests reported from different laboratories. Various devices have been employed to improve the readings. In some methods the microscope is used; in others, special lamps or lenses to detect the finer degrees of flocculation.

The Laughlen test for syphilis was developed by Dr. G. F. Laughlen of Canada and reported by him in August 1935.2 One of the chief advantages of this test is the greater ease of reading the results as greater visibility has been secured by the addition of a water-insoluble stain which colors or adheres to the suspended particles of the antigen but does not color the liquid in which they are suspended, thus affording a good contrast when agglutination of these particles occurs. The antigen for this method is prepared in the same way as the Kahn antigen. cholesterolized, and then modified by adding Scarlet R stain and tincture of benzoin compound. It is then diluted with 1.5 per cent saline which produces an inactive stable emulsion. At this point considerable experience is necessary to modify and dilute the antigen properly and adjust it to the proper sensitivity. It must be adjusted to react in one minute with strongly positive seraand remain free from agglutination for at least ten minutes with negative sera. Laughlen suggests that the antigen be made in large quantities only by those thoroughly trained in the technic, and dispensed from a central laboratory. Directions for the preparation of this antigen are outlined in Laughlen's article. The antigen for the tests reported here was purchased in the inactivated form from the Lederle Laboratories together with the 10 per cent NaCl to activate it. The amount of 10 per cent NaCl necessary to activate one cc. is marked on each lot.

The antigen and the sodium chloride are measured with a one cc. pipette graduated in hundredths, a separate one for each. Measurements must be absolutely exact to assure proper activation and not less than one cc. of antigen should be activated for, as is well known, the smaller the quantity of any reagent prepared, the greater will be the error. The small bottle for the antigen (of not greater than 3 cc. capacity) is washed chemically clean and dried with alcohol and ether. The stopper must be covered with lead foil. The inactivated antigen is stable for about four to six weeks, the activated antigen will keep from seven to ten days. (The Lederle Laboratories say that activated antigen stored in pyrex tubes will remain useable for as long as four weeks. We have not tested this claim.) One cc. of antigen suffices for approximately seventy-five tests. The antigen must be activated twenty-four hours prior to its use. We activate antigen once a week in a quantity sufficient to last one week so that it is always available and ready for use. Activated antigen on standing shows a

deep pink precipitate and pink supernatant fluid. In 18 to 24 hours after activation nearly complete sedimentation has occurred, indicating that it is fully active and ready for use. Complete clearing in a shorter time indicates over-activity of the antigen and it should not be used. It must be thoroughly shaken just before use.

In performing individual tests the ordinary microscopic slides can be used. A black wax pencil is used for marking slides as it washes off more easily than the other colors. Red wax pencils should not be used as the red particles may mix with the serum-antigen mixture and cause confusion in interpreting the results. The microscopic slide is divided into three parts with the wax pencil. the patient's serum placed on the right hand side, the four plus control on the left hand side and the negative control in the center. When individual tests are run, it is essential to have both a positive and a negative control. When running large series of tests, only a positive control need be used as there are certain to be negative sera in the series. For running large series of tests we use the micro slides for making milk smears according to the Breed and Brew method. These slides are 115 mm, long, 52 mm, wide, somewhat thicker than an ordinary micro slide and have an etched strip running lengthwise along the edge of each side on which to place the sera numbers with wax pencil. These slides are one dollar per dozen. (Ordinary glass may be cut this size or larger and etched in a similar manner.) These slides are divided into ten parts with a black wax pencil so that ten tests can be performed simultaneously. Thus, it can be seen that the slide for ten Laughlen tests is more quickly and easily prepared than the rack of thirty tubes for ten Kahn tests or the paraffin ringed slides for the Kline tests and the slide is also quickly and easily cleaned. Both of these items are important from the point of time and labor saving. With an assistant to rotate one of the slides, twenty tests can be completed in about fifteen minutes. I do not believe it wise to run more than ten tests on a slide as too much time would elapse between the adding of the antigen and the mixing of the serum-antigen mixture. The same principle holds in the Kahn method. If two racks of tubes (10 tests to each) are used. Kahn advises that to save time and prevent the antigen from evaporating an assistant add the serum to the antigen in the first rack while an assistant adds antigen to the second rack.

Two ordinary dropper pipettes with bulbs attached are used. The one for the antigen should be drawn out so that the tip is approximately one mm. in outside diameter; the pipette for the serum is drawn out so that the inside diameter at the tip is one mm., as the quantity of serum should be slightly greater than the quantity of antigen. Great care should be exercised in making these pipettes and several extra ones should always be on hand, as in the technic as given by Laughlen there is great danger that the serum-antigen proportions would not be kept constant which would be certain to lead to inaccuracies in the results. The same two pipettes, made according to the directions given, were used throughout the experiments to be reported. Another important point is the

angle at which these pipettes are held in dropping the antigen and serum as the size of the drop can be altered thereby. The antigen pipette is held *vertically*, the serum pipette at an angle of about 45 degrees. The antigen pipette is cleaned by running alcohol and ether through it. The pipette for the serum is cleaned as any pipette used for sera.

When using the Breed and Brew micro slides, a drop of patient's serum (fresh, inactivated) is placed in each square (properly numbered), rinsing the pipette thoroughly between each serum with physiologic NaCl. To avoid adding physiological NaCl to the test mixture, draw the serum in and out of the pipette once before taking out the serum for the test. The sera are measured out first, as drying of the sera will not interfere with the results. The activated antigen is shaken and a drop added to each drop of serum. Note the time and quickly mix each drop of serum-antigen mixture with a tooth pick using a separate tooth pick for each serum. The slide is then rotated as for typing blood and observed macroscopically by indirect light, holding the slide a little to one side of a shaded bright light so that the light shines on it. The tests are observed best over a black surface. The observer's eves are above the shaded light and looking through the slide at the black surface of the laboratory table. Do not look directly at the light. The final results are read at the end of ten minutes, the slide being rotated for this length of time. Constant rotation is of extreme importance.

A positive reaction is recognized by the macroscopic appearance of large, coarse, distinctly red particles which rapidly increase in size. A strongly positive reaction is apparent between one and five minutes. Weakly and strongly positive sera usually give the same amount of agglutination or clumping at the end of ten minutes. The time required for the reaction to become visible is the index of the degree of positiveness of the specimen. Occasionally a weakly positive serum, especially that from a treated case, will not give as complete clumping in that the coarse agglutinated particles are smaller, but if it is a positive serum the particles will be distinctly reddish in color and the mixture will contain very little, if any, milky fluid. A weakly positive reaction becomes apparent between five and ten minutes. When the reaction is typically negative, the pink fluid remains homogeneous and milky as at the beginning of the test. An occasional serum will show coarse or fine light pink granules in a pink milky fluid. This is a negative result. In my series I called many of these results doubtful as I was not sufficiently familiar with the interpretation but they were shown to be negative by the Kolmer and other methods. Occasionally a smooth ring or rim of deep pink will dry around the edge of the pink granular milky fluid. This is also a negative result. When difficulty arises in reading a negative, as such, it is probably due to the activated antigen becoming oversensitive and a fresh lot of activated reagent should be made. It is advisable to prepare a new lot of activated antigen a day or two in advance of the time it is likely to be required. The new lot can be used to check positive or doubtful readings

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secured with the older reagent. It is also advisable to keep the older reagent on hand a few days to check weak positive and doubtful sera. This old activated antigen makes a splendid "presumptive test" preparation.

Results should be reported simply as strongly positive, weakly positive, doubtful or negative. The archaic system of reporting by plus marks is confusing, meaningless and should be abandoned.

Laughlen² reporting on 400 specimens taken for routine examination found 98 per cent agreement with the Wassermann test and 99 per cent agreement with the Kahn test. Sera from 118 patients receiving anti-syphilitic treatment showed 93.5 per cent agreement with the Wassermann and 97 per cent agreement with the Kahn.

Robinson and Stroud³ reporting on 1000 routine sera found 93 per cent agreement with the Wassermann test and 97 per cent agreement with the Kahn test.

In comparing the Laughlen test with the Kline test* on 1000 routine sera I found 95.6 per cent agreement. There were 639 negative agreements; 317 positive agreements, and 44 disagreements. In comparing the Laughlen test with the Kolmer-Wassermann test† on 1100 specimens I found 95.7 per cent agreement. There were 792 negative agreements; 260 positive agreements; and 48 disagreements.

The above reports by Laughlen, by Robinson and Stroud, as well as this report, are not completely enlightening as the sensitivity and specificity of the Laughlen test are not determined. For this it would be necessary to have the histories on these patients in order to know if they were syphilitic or non-syphilitic.

Lacking the necessary clerical assistance to read carefully the histories on all of these patients, I confined my history reading to the cases on which there was a disagreement between tests as I felt that this would give some idea of the sensitivity of the test. The results of history reading were far from satisfying as many cases were not the type that would call for special study and a search for signs of syphilitic infection. Some were accident cases and treated only for injuries. In the 92 histories investigated it was necessary that 39 patients be listed under the heading "No syphilitic history obtained;" 53 patients had a definite syphilitic history.

The 44 disagreements between the Laughlen and Kline tests can be best shown as in tables 1 and 2.

It is quite likely, from the results obtained, that six of the eleven patients listed as positive in table 2 were syphilitic as Dr. Kolmer has said that a strongly positive Wassermann reaction is indicative of an active syphilitic process,

^{*} See note at end of article.

[†] The Kolmer-Wassermann is the routine test at Stuart Circle Hospital. This series includes the 100 specimens received from the University of Virginia.

whether the physician is able to find it or not. The six patients in this group who had a positive Laughlen test were also positive by one, two or all three of the other methods. The histories, also, were suggestive of syphilitic infection.

The 48 disagreements between the Laughlen and Kolmer-Wassermann tests were as shown in tables 3 and 4.

TABLE 1
TWENTY-THREE SPECIMENS FROM SYPHILITIC PATIENTS

	POSITIVE	NEGATIVE	DOUBTFUL
Laughlen	20 (86.9%)	2	1
Kline	3 (13.0%)	6	14

TABLE 2
TWENTY-ONE SPECIMENS "NO SYPHILITIC HISTORY OBTAINED"

	POSITIVE	NEGATIVE	DOUBTFUL
Laughlen	11	9	1
Kline	9	3	9

TABLE 3
THIRTY SPECIMENS FROM SYPHILITIC PATIENTS

	POSITIVE	NEGATIVE	DOUBTFUL
Laughlen	22 (73.3%)	4	4
Kolmer	8 (27.5%)	12	10

TABLE 4
EIGHTEEN SPECIMENS "NO SYPHILITIC HISTORY OBTAINED"

	POBITIVE	NEGATIVE	DOUBTFUL
Laughlen	6	6	6
Kolmer	9	7	2

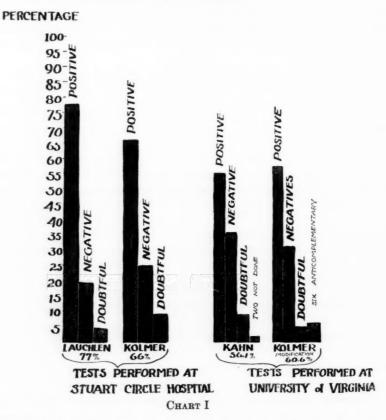
In a series of 650 presumably non-syphilitic patients at Stuart Circle Hospital with various diseases and surgical conditions there was one false positive result giving a specificity of 99.3 per cent in this series.

From the histories studied there were 53 patients with a syphilitic history. In this group there were 6 negative Laughlen tests or 11.3 per cent missed. This would give a sensitivity of 88.3 per cent in this small group which compares most favorably with other methods.

It is readily recognized that in these series it was impossible to determine accurately the sensitivity of the Laughlen test without a group of known syphilitic patients. Dr. D. C. Smith, Head of the Department of Dermatology of the University of Virginia, therefore furnished me with 100 blood specimens

SENSITIVITY of LAUGHLEN TESTS

100 SERA FROM KNOWN SYPHILITIC PATIENTS FROM THE DEPARTMENT OF DERMATOLOGY UNIVERSITY OF VIRGINIA



from known syphilitic patients, ninety of whom had had various amounts and kinds of treatment; ten had not yet received treatment. On these specimens I performed both Laughlen and Kolmer tests and the University of Virginia laboratory performed Kahn and Wassermann tests on the same specimens. The results are shown in chart I.

While working on this study I had the impression that the Laughlen test would prove to be too sensitive, and that I was obtaining a high percentage of false positive results. However, in the final checking there were positive Laughlen tests on 17 patients in the series of 2000 who did not have a definite syphilitic history. This would give a specificity of 99.2 per cent and as previously stated, it is believed that six patients in this group were syphilitic.

It has also been calculated, from the results obtained, that if a serum is negative by the Laughlen test, in 99.4 per cent of cases it will be negative by any other test. This is as reliable as any test so far devised. It can, be used therefore, as a routine test and all doubtful and positive results checked by another reliable method in accord with the recommendation of the committee on the evaluation of serodiagnostic tests for syphilis4 which is as follows: "There is some evidence that a properly performed, highly sensitive flocculation test might be used as a routine for the purpose of excluding the likelihood of syphilis. If a negative result is obtained by such a method, it is quite likely that it will be negative by any other method. If the test yields a positive result it should be repeated and compared with one or more highly specific flocculation or complement fixation tests." Doubtful results by any method should be repeated and checked by another method. In the complement fixation methods, tests that are slow in clearing, plus-minus readings, prezone reactions, anticomplementary results, should be treated in the same way.

Considerable experience is necessary to properly and accurately interpret the results in the Laughlen test and any one desiring to use this method should first compare the results with the results of several hundred tests by a method with which he is familiar so as to learn to interpret the results properly. Certainly anyone acquainted with the complexities of serological reactions appreciates this. The granular reactions and reactions due to drying give the most concern. There will be one or two per cent of these. The beginner will be inclined to interpret these reactions as weakly positive. In the granular reactions the clumps are pink and not red as in a positive test. The safest method is to

interpret these results as doubtful, repeat the test and check by another method. An indefinite or doubtful result, if the patient has been previously treated, may mean that syphilis is still present. When there is difficulty in reading a negative result as such, it is probably due to the activated antigen becoming oversensitive, and a fresh lot should be made. In performing the Laughlen test, the operator must be alert to insure the use of only sensitive, reliable, active antigen. From my experience I would say that, like all other serologic tests for syphilis, considerable experience and skill are required, as well as a thorough understanding of serology in general, to properly perform the test and interpret the results. Interpretation is the most important phase of any test. I do not believe that this test should be left to the average intern or technician unless performed under the supervision of an experienced serologist, just as are other tests for syphilis.

It is unfortunate that the small pamplet, which accompanies the antigen for this test, contains so many inaccurate and misleading statements. It is stated, for example, that this test may be performed on fresh uninactivated sera and this statement is also made by Laughlen. Evidently this problem was not sufficiently investigated. In a series of uninactivated strongly positive (4+) sera I found that 40 per cent of the specimens gave negative results; 10 per cent doubtful results, and only 50 per cent were positive, while, when the same sera were inactivated for from seven and one-half to fifteen minutes, they gave strongly positive results. Sera inactivated for thirty minutes would occasionally give weakly positive results, while after fifteen minutes inactivation the same sera were strongly positive. For these reasons, I advise that the sera for this test be inactivated at 56°C. for from eight to fifteen minutes. Reactions occur more quickly if the sera are chilled in the icebox just before the tests are run. Sera should be free of bacterial contamination and should be reasonably fresh. The presence of a large amount of bile in the serum may interfere with the test as the bile obscures the color of the dve. Cord blood sera containing a large amount of mucous from the cord may show a

curdling effect and interfere with reading the results. Citrated or oxalated plasma should not be used as a small percentage of positive plasmas will give negative or doubtful results.

It is also stated by the pamphlet and by Laughlen that spinal fluid can be tested by this method, using the same technic as used for blood. A group of positive spinal fluids were tested by the Laughlen method and all were negative. I suggested to Dr. Laughlen that I felt that the syphilitic reagin in the spinal fluid should be concentrated by some method before testing and he advised that spinal fluid be concentrated as for the Kahn test. We expect to run a series of spinal fluids which will be reported at a later date.

The pamphlet also states: "As it (the Laughlen test) resembles the method employed in blood grouping, hospital interns and technicians are familiar with the technic." The only way in which it resembles the method employed in blood grouping is in the rotating of the slide. Certainly, the hospital orderly could perform this part of the test, but just because one can rotate a slide or shake a tube would not make him competent to perform a Laughlen test, interpret the results, or supply the skill and experience essential to escape disaster.

This test requires less time in the preparation of materials (test slides) and in the cleaning of the apparatus; results on the majority of sera are easier to read than results on other flocculation tests but, the most meticulous attention to various essential details and a thorough understanding of serology in general are required to obtain safe and reliable results. It would be difficult, if not impossible, to outline all of the difficulties and pitfalls to be avoided in performing this test, just as it would be to do this for any other serological test. An experienced serologist instinctively avoids these pitfalls or knows how to deal with them as they arise due to his experience and skill and his intimate acquaintance with the complexities of the serology of syphilis. If this test, or any other test for syphilis, is placed in the hands of interns, technicians or office assistants, I would not want my blood to be one of the specimens under consideration. It is earnestly hoped that all future information concerning this test will eliminate ill-advised and misleading statements.

CONCLUSIONS

The Laughlen test for syphilis is less time-consuming than other flocculation tests and the results are easier to read (not interpret). It is of value in following the response of sera of patients under treatment; for testing donors for emergency transfusions, and for pre-operative cases.

The Laughlen test requires as much experience and skill in its performance and interpretation and the same meticulous attention to various essential details as other flocculation tests and should be performed under the supervision of an experienced serologist.

In comparing the Laughlen test with 1000 Kline tests, 1100 Kolmer-Wassermann tests and 100 Kahn tests, the Laughlen test was shown to be just as specific and a little more sensitive than the other tests as performed in this study.

The Laughlen test is suitable as a routine test, the positive and doubtful results to be checked by another reliable test.

The Laughlen test has not yet been shown applicable to the testing of spinal fluids.

The thanks of the author are tendered Dr. J. H. Scherer of the Medical College of Virginia for permission to perform the Laughlen test in their laboratory and compare the results with their Kline test and Kolmer modification of the Wassermann. Thanks are also tendered to Dr. L. E. Jarrett and Dr. F. J. Wampler of the Medical College of Virginia for permission to check the patients' histories. The author is also indebted to Dr. D. C. Smith and Dr. J. M. Hitch of the University of Virginia for furnishing blood specimens and histories on 100 syphilitic patients.

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A RELIABLE, SENSITIVE, SIMPLE, AND RAPID SLIDE FLOCCULATION TEST FOR SYPHILIS*

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The evaluation of original serodiagnostic tests for syphilis conducted by the U. S. Public Health Service and the American Society of Clinical Pathologists in 1934–1935, and the subsequent studies made in state laboratories indicate that the goal of ideal sensitivity is still unattained. It is agreed that 100 per cent sensitivity and 100 per cent specificity in a single test is practically impossible. There is a difference of opinion just where the optimum ratio for practical purpose lies; but the ideal goal of any laboratory procedure is maximum sensitivity without loss of specificity.

During the last few years flocculation tests have rapidly gained favor, chiefly due to their relative simplicity and economy in contrast to the complexity and high cost of complement fixation tests. Any flocculation test, to be of real value as an aid in the diagnosis of syphilis, must possess specificity and sensitivity in a high degree, should be quickly and easily performed and should produce clear-cut results. A new modification of the slide flocculation test which has been developed in the serologic division of the Indiana State Board of Health, and which conforms with these requirements, will be presented in this paper. The application of the test to spinal fluid and other new improvements will be described in another paper.

REAGENTS AND TECHNIC

Preparation of the antigen extract

1. 20 grams dehydrated beef powder (Difco Laboratories), 10 grams powdered egg yolk† and 200 c.c. ether (Mallinckrodt for anesthesia) are placed in a

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^{*} Received for publication November 7, 1938.

[†] Powdered egg yolk may be purchased at any reliable wholesale dairy supply company, or from Bessire & Co., Inc., Indianapolis.

500 c.c. wide mouth, glass stoppered bottle. The mixture is shaken in a mechanical shaker for 5 minutes, or by hand for 15 minutes.

2. Filter through a 24 cm. paper (Schleicher & Schull *597) into a 500 c.c. flask. Repeat the ether extraction four additional times using 100 c.c. of ether each time. Use a new filter paper for each filtration and collect all the ethereal filtrates in the 500 c.c. flask. The combined filtrates will be used in step 6.

3. After the last extraction is completed spread the moist powder on a new piece of filter paper. Place the powder in a 37°C. incubator for about 15 minutes

to remove the ether adhering to the powder.

4. The dry powder is then placed in a 500 c.c. glass stoppered bottle, 80 c.c. absolute ethyl alcohol is added and the mixture shaken in a mechanical shaker for 4 hours, or the mixture is left at room temperature for 3 days, being shaken for 5 minutes three times each day.

5. Filter through a 24 cm. paper (Schleicher & Schull #597) into a 100 c.c. wide mouth, glass stoppered bottle. Discard the powder.

6. The combined ethereal filtrates are now placed in a large evaporating dish and the ether evaporated off by placing the dish in a water bath at 55°C.

- 7. The concentrated ether extracts are then poured rapidly into an evaporating dish 8.5 inches in diameter containing 100 c.c. of acetone (Merck's Reagent) which has previously been warmed to 55°C. Immediately decant the acetone into two 50 c.c. centrifuge tubes, centrifuge at about 2000 R.P.M. for 5 minutes. Pour off and discard the acetone. Collect the acetone insoluble lipoids from the tubes with a spatula and add them to the alcoholic extract obtained in step 5. Place the bottle in a 55°C. water bath for 30 minutes, shaking at frequent intervals. Allow the extract to cool by placing the bottle in the refrigerator for 30 minutes.
- 8. Filter through a 12.5 cm. paper (Whatman #40). The antigen is now ready for titration and will be referred to as the stock antigen. Any precipitate appearing after the extract has stood should be removed by filtration. The antigen is kept at room temperature.

Preparation of the 1 per cent cholesterinized alcohol

Place 500 mg. C.P. cholesterin (Pfanstiehl) and 50 c.c. absolute ethyl alcohol in a 100 c.c. glass stoppered bottle. Heat in the water bath at 55°C. for about 30 minutes, or until the cholesterin has completely dissolved; shake at frequent intervals. Filter the solution.

Preparation of the buffered saline solution

A buffered saline solution having a pH 6.3 to 6.4 and a salt concentration of 10 grams per liter is prepared as follows:

Sodium Chloride	2.025 gm.
Secondary Sodium Phosphate (Na ₂ HPO ₄)	0.425 gm.
Primary Potassium Phosphate (KH ₂ PO ₄)	

Double Distilled Water	250.00	cc.
N/1 Hydrochloric Acid	0.8	cc.
Formaldehyde (Merck's Reagent)	0.25	cc.

The saline solution is filtered and kept in a glass stoppered bottle; it is not necessary to prepare it more often than once a month. It is, however, necessary to filter the solution as often as there is a sediment because particles of dirt or other debris which may be transferred to the suspension will appear in the field of vision during the reading of the test and, unless differentiated from specific flocculate, may lead to a false reading.

Titration of the antigen

Titration of any antigen is necessary because it is impossible to obtain extracts of uniform antigenic value. Once the antigen has been titrated to the desired sensitivity, however, the routine technic for the preparation of the suspension is simple and rapidly done.

Determination of the optimum lipoid-cholesterin ratio

- Set up 5 clean and dry serological test tubes in a rack. Label them from 1 to 5.
 - 2. Place 0.1 c.c. of the stock antigen directly into the bottom of each tube.
- 3. Add 0.9 c.c., 1.4 c.c., 1.9 c.c., 2.4 c.c., and 2.9 c.c. of 1 per cent cholester-inized alcohol to each of the five tubes respectively. Cork the tubes and mix thoroughly the contents of each tube. This will give a ratio of 1:10 in tube #1, 1:15 in tube #2, 1:20 in tube #3, 1:25 in tube #4, and 1:30 in tube #5.
- 4. Take five 20 to 30 c.c. bottles and label them 1:10, 1:15, 1:20, 1:25, and 1:30 respectively.
- 5. Place 3 c.c. of the buffered saline solution directly into the bottom of each of the five bottles.
- 6. With a 1 c.c. pipette graduated to the tip measure 0.4 c.c. (reading from the bottom of the pipette) of the 1:10 cholesterinized antigen; hold the bottle in the left hand and impart a rapid rotating motion to it as the antigen is being discharged directly and at *once* into the saline from the pipette held in the right hand. The bottle is corked and shaken for 10 seconds; then it is allowed to stand at room temperature without further manipulation.
- 7. Proceed in the same manner in preparing the 1:15, 1:20, 1:25, and 1:30 antigen suspensions. When all five suspensions have been prepared they are allowed to stand at room temperature from 3 to 4 hours. Although the suspension for best results should be allowed to stand from 3 to 4 hours to permit it to reach its optimum sensitivity, it may be used in emergencies within 15 minutes after preparation by placing the bottle containing the freshly prepared suspension in the refrigerator at 6°C. to 8°C. for 15 minutes. However, at this stage of ripening it will occasionally fail to react with weakly positive serum.

Trial of the suspensions

1. At the end of 3 to 4 hours draw up suspension #1 (ratio 1:10) into a 5 c.c. syringe fitted with a 25-gauge needle.

2. Select 30 or more sera from cases known to be free from syphilitic infection. Extreme care must be taken that no serum is included which may contain even a few reacting units.

3. Place 0.05 c.c. of serum from each of the 30 negative specimens into the corresponding one of the thirty chambers of three glass slides which have been placed on a slide holder. (See paragraph on Preparation of Slides.)

4. Discharge one drop (by exerting slight pressure on the piston of the syringe with the index finger) of the 1:10 suspension into each of the 30 sera.

5. Rotate the slides with a circular slightly "jerky" motion for 4 minutes at 120 rotations per minute. It is important that the number of rotations be that indicated, and that the proper motion be given to the slides to insure that the antigen particles become well dispersed throughout the area of the rings. It is not necessary that the motion be of such nature as to cause the sera to "jump" the rings.

6. Examine each of the rings under the low power objective of the microscope with subdued light. Record the results. Every one of the rings should show numerous, very small, round or slightly elongated particles of lipoid-cholesterin complex. These particles uniformly dispersed throughout the field should not show the slightest clumping.

7. Place three other slides on the holder and using the same sera as previously used proceed to try out the 1:15 suspension. Follow this with the 1:20, the 1:25, and finally with the 1:30 suspension. Pipetting of the sera should be carried out as quickly as possible because evaporation takes place rapidly due to the small volume used.

Usually within the range of 1:10 to 1:30 will be found one or more ratios in which the cholesterin is in excess of the lipoids, allowing the spontaneous clumping of the particles. Obviously these ratios can not be employed in the test proper since false positive reactions will be obtained with such suspensions.

Determination of the antigenic quality of the suspension

Having already determined the lipoid-cholesterin ratios which will not cause false positive reactions with negative sera, the next step in the standardization of the antigen is the evaluation of the antigenic properties of these suspensions. For this purpose select at least 10 partially positive sera, preferably those from long treated cases. The object is to employ sera containing as few reacting units as possible.

1. Place 0.05 c.c. of serum from each of the ten partially positive specimens into the corresponding one of the ten chambers of a glass slide.

2. Discharge one drop of the 1:10 suspension into each ring. Rotate the

slide for 4 minutes at 120 rotations per minute. Examine through the microscope. Record the results.

3. Proceed to try out the rest of the suspensions which were found to give clear-cut negative reactions with known negative sera by following the same procedure as for the 1:10 suspension. When the trials are conducted with sera containing relatively few reacting units it will be observed that the lower the lipoid-cholesterin ratio the weaker the reaction (clumping); the flocculate increases in size as the ratio increases.

After recording all the results and having made a study of them, a final lipoid-cholesterin ratio is selected which is the titer of the antigen. For maximum sensitivity, the suspension containing the highest lipoid-cholesterin ratio which does not cause the least clumping in the presence of negative sera is selected as the titer. If a less sensitive antigen suspension is desired a lower ratio is chosen. Naturally, a greater degree of safety is obtained by using a lower ratio but the sensitivity usually will be decreased.

When the titer of the antigen has been determined a sufficient amount of cholesterinized antigen is prepared to meet the individual need for approximately one month. Example: If the ratio selected is that of 1:20, then by taking 0.5 c.c. of the stock antigen and adding 9.5 c.c. of the 1 per cent cholesterinized alcohol, a supply for about one month is obtained, since 0.4 c.c. of the cholesterinized antigen, regardless of the ratio, is the fixed amount to use in 3 c.c. of buffered saline solution. This volume (3.4 c.c.) will be sufficient for about 300 tests.

It would seem that the antigenic determination could be eliminated and the titer be based solely on the lipoid-cholesterin ratio. By this procedure less time and labor would be involved in the standardization of the antigen. However, actual experience has shown that this determination is very desirable if not essential. Sometimes it can be demonstrated that two different ratios give approximately the same degree of clumping with weakly positive sera, and while there is little choice between the two, the logical dilution to employ is the one having the lower ratio, because the sensitivity is the same while the margin of safety (specificity) is increased. Obviously if the antigenic determination had not been carried out the dilution of choice as determined by the lipoid-cholesterin ratio titration would be the higher of the two. It is possible to eliminate all titrations and to set an arbitrary mean which past experience has shown is the average lipoid-cholesterin ratio. For instance, the most frequently encountered ratios which give clear-cut negative reactions with negative sera are the 1:10, 1:15, 1:20 and 1:25. Therefore, an arbitrary selection of the dilution containing the 1:15 or 1:20 ratios could be made provided strict adherence to details of technic is observed. I believe, however, that both titrations are absolutely necessary if the highest sensitivity consistent with safety is to be obtained. I also believe that in general the accuracy with which serologic reagents are standardized largely determines the quality of work performed in any laboratory irrespective of the merits of the technic involved.

Patient's serum

The patient's serum is separated from the clot by centrifugalization and heated for 30 minutes in the water bath at 55°C. to 56°C. For emergency pretransfusion test the serum may be inactivated at 60°C. for 10 minutes. Inspection of sera for visible precipitate after heating should be done as a matter of routine. Occasionally heated serum contains a precipitate which, if not removed, may interfere with serologic reactions in general. There is not, however, the possibility that the precipitate, if left in the serum, will be mistaken for the true flocculate of a positive reaction when read microscopically, which does occur when specimens containing this pseudo-precipitate are read macroscopically.

Preparation of the ringed glass slides

The technic followed in making the instrument and rings is that described by Kline¹ with the exception that melted sealing wax is substituted for paraffin. The wax rings are made by dipping the instrument into the hot wax, draining quickly at one point and transferring the remainder to a glass slide, 2 x 3 inches, which has previously been cleaned and made fat free. After the wax has been allowed to harden, a second and third layer may be applied if the chambers are not sufficiently deep. For convenience only ten rings are made on each slide.

Care of the slides after use

The wax rings should not be removed from the glass slides after use. Immediately after reading the tests the slides are placed in a metal slide holder which is kept immersed in distilled water. By this procedure drying of sera on the slides is prevented. After the completion of the tests, the slides are scrubbed with a hand brush and soapy water; they are thoroughly rinsed in tap water and finally rinsed in distilled water. The slides are then transferred to a slide box and allowed to dry at room temperature under cover, or they may be dried by rubbing the chambers of the rings with a soft cloth free from lint. If proper care is given the slides, they may be used indefinitely.

The test proper

Once the desired sensitivity of the antigen has been determined the daily routine technic for the preparation of the suspension is very simple and quickly done. The reagents for the test proper have been reduced to two solutions: buffered saline solution and cholesterinized antigen solution.

The routine technic is as follows:

- 1. Place 3 c.c. of buffered saline solution directly into the bottom of a 20 to 30 c.c. bottle.
- 2. With a 1 c.c. pipette graduated to the tip measure 0.4 c.c. (reading from the bottom) of the cholesterinized antigen; hold the bottle in the left hand and impart a rapid rotating motion to it as the antigen is being discharged directly and at *once* into the saline solution from the pipette held in the right hand.

The bottle is corked and shaken for 10 seconds, then it is allowed to stand at room temperature from 3 to 4 hours, at which time the suspension reaches its optimum sensitivity, or the bottle may be placed in the refrigerator at 6°C. to 8°C. for 15 minutes to accelerate the ripening of the antigen suspension and then used immediately. The suspension continues to be usable for 24 hours after which it decreases in sensitivity; therefore, dependable results require that it should be used within this period of time.

3. At the end of 3 to 4 hours at room temperature or 15 minutes in the refrigerator the suspension is transferred to a 5 c.c. glass syringe fitted with a

25-gauge needle and is then ready for instant use.

4. Place one, two or three ringed glass slides on a slide holder, depending on the number of specimens to be tested. Place 0.05 c.c. of each patient's serum, which has previously been heated for 30 minutes at 55°C. to 56°C., into one of the ten chambers of the glass slide. Discharge one drop of the ripened antigen suspension into each of the chambers. When the number of specimens tested is small include known negative and positive sera as controls on the antigen.

5. Rotate the slide holder for 4 minutes at 120 rotations per minute.

6. First examine every one of the rings macroscopically to make certain that no serum has "jumped" the ring and contaminated another. Then examine them microscopically under the low power objective with subdued light. Inspection of the periphery of the rings for clumps should be made a routine practice, for occasionally the clumps are very compact and have a tendency to locate in the outer portion of the ring. Record the results as follows:

No clumping: negative; very small clumps: 1 plus; small clumps: 2 plus; medium size clumps: 3 plus; large clumps: 4 plus. An alternative method of

reading may be used as follows:

No clumping: negative; very small to small clumps: doubtful; medium to

large clumps: positive.

In reading weakly positive reactions care should be taken to differentiate red blood cells or debris—which may be contained in the serum, or on the slide, or in the antigen suspension—from the true flocculate of a positive reaction. This caution obviously applies only in weakly positive reactions since in strongly positive ones debris or blood cells are masked by the large clumps of the reaction. The reading of any serological test requires judgment and experience since no accurate standard can be prepared. Although no experience is needed to read strongly positive reactions, time and observation alone will lead to correct interpretation of the weakly positive reactions.

COMMENT

The principles involved in this modification do not represent a radical departure from those which have previously been established by others in the field of serology, nor do the ingredients entering into the preparation of the antigen extract and antigen suspension differ basically from those which are used in nearly all of the flocculation tests. The explanation for the high degree of sensitivity and specificity may be found in the lipoid-cholesterin ratio; the hydrogen ion concentration of the suspension; the serum-antigen ratio; and the use of egg yolk.

An exhaustive study has been made of results obtained in over 100,000 specimens tested with this modification and three other widely used tests during the past three years. These results clearly demonstrated that the new modification possesses a greater degree of sensitivity in general, and especially in early, latent, and neurosyphilis, and in patients under antisyphilitic treatment.

To test further the reliability of the antigen, an unofficial study was made with specimens furnished by the Public Health Service for the 1936–1937 evaluation survey; this study proved in a convincing manner its potential value. The technique was then officially entered in the 1938 evaluation survey. The rating received by this modification was 86.7 per cent sensitivity and 100 per cent specificity. (Ratings of other tests are given in table IV.)

Although the results obtained with this technic have been very satisfactory, no claim is made: that this test will detect every case of syphilis; that it will exclude syphilis if the reaction is negative; that every weakly positive reaction means syphilitic infection; that the size, shape or number of particles of the flocculate indicates a particular stage of the disease; that the test can or should be done in the physician's office; nor that it can or should be done in laboratories where facilities for proper standardization and control are not available.

Elimination of certain unsatisfactory features of some flocculation tests has been accomplished with this method. It excludes or reduces prolonged incubation (or a combination of incubation and centrifugalization), uncertainty in obtaining uniformity of extracts and suspensions, and finally difficulty in reading the reaction. In addition, economy of time and materials and ease of preparing the reagents will be found to be important features of the test.

A detailed analysis of the relative value of this modification of

TABLE 1

		TED PRIM GROUP I,			TREATED SECONDARY STPHILIS (ONE CASE UNTREATED) GROUP II, (16 CASES)				
TECHNICS	Speci- mens exam- ined	Doubt- ful reports	tive	Per- centage of positive reports	Speci- mens exam- ined	Doubt- ful reports	Posi- tive reports	Per- centage of positive reports	
Eagle C.F	19	3	3	15.8	15	0	8	53.3	
Eagle M.F		0	5	27.7	13	0	6	46.1	
Hinton	17	0	7	41.2	16	0	11	68.7	
Kahn Standard	19	0	3	15.7	14	0	8	57.1	
Kahn Presumptive	19	0	3	15.7	14	0	9	64.2	
Kline Diagnostic	18	0	3	16.6	15	0	9	60.0	
Kline Exclusion	18	3	5	27.8	15	1	9	60.0	
Kolmer C.F	19	0	4	21.1	16	0	9	56.2	
Mazzini	18	3	6	33.4	15	1	10	66.7	

TABLE 2

	(01	ED CONGI NE CASE U ROUP III	INTREAT	ED)	TREATED AND UNTREATED LATENT SYPHILIS GROUP IV, (75 CASES)				
TECHNICS	Speci- mens exam- ined	Doubt- ful reports	tive	Per- centage of positive reports	Speci- mens exam- ined	Doubt- ful reports	Posi- tive reports	Per- centage of positive reports	
Eagle C.F	5	0	4	80	74	5	59	79.7	
Eagle M.F	5	0	5	100	73	5	60	82.1	
Hinton	4	0	4	100	71	1	63	88.7	
Kahn Standard	5	1	4	80	73	5	52	71.2	
Kahn Presumptive	5	0	5	100	73	1	62	84.9	
Kline Diagnostic	5	1	4	80	73	9	53	72.6	
Kline Exclusion	5	0	5	100	73	5	65	89.0	
Kolmer C.F	5	0	5	100	75	0	63	84.0	
Mazzini	5	0	5	100	74	1	70	94.6	

TABLE 3

	2	TERTIARY	SYPHILI	В	TREATED AND UNTREATED NEUROSYPHILIS GROUP VI, (42 CASES)				
TECHNICS	Speci- mens exam- ined	Doubt- ful reports	tive	Per- centage of positive reports	Speci- mens exam- ined	Doubt- ful reports	Posi- tive reports	Per- centage of positive reports	
Eagle C.F	50	2	43	86.0	42	0	31	73.8	
Eagle M.F	49	0	47	95.9	42	0	32	76.2	
Hinton	50	1	47	94.0	41	1	35	85.4	
Kahn S	49	0	47	95.9	40	3	27	67.5	
Kahn P	49	0	47	95.9	40	1	32	80.0	
Kline D	50	0	48	96.0	42	3	32	76.2	
Kline E	50	0	48	96.0	42	2	37	81.1	
Kolmer C.F	50	0	48	96.0	42	1	33	78.6	
Mazzini	50	0	48	96.0	42	1	38	90.5	

TABLE 4
Total Cases of Syphilis, Groups 1, 2, 3, 4, 5, and 6 (207 Cases)

TECHNICS	SPECIMENS EXAMINED	DOUBTFUL REPORTS	PERCENT- AGE OF DOUBTFUL REPORTS	POSITIVE REPORTS	PERCENT- AGE OF POSITIVE REPORTS	NUMBER OF SPECIMENS NOT TESTED
Eagle C.F	205	10	4.9	148	72.2	2
Eagle M.F		5	2.5	155	77.5	7
Hinton	199	3	1.5	167	83.9	8
Kahn S	200	9	4.5	141	70.5	7
Kahn P	200	2	1.0	158	79.0	7
Kline D	203	13	6.4	149	73.4	4
Kline E	203	11	5.4	169	83.3	4
Kolmer C.F	207	1	0.5	162	78.3	0
Mazzini	204	6	2.9	177	86.8	3

TABLE 5

CASE NO.		EAGLE C.F.	EAGLE M.F.	HINTON	KAHN 8.	KAHN P.	KLINE D.	KLINE E.	KOLMER C.F.	MAZ- ZINI
				Lat	ent sy	philis				
59	Treated	_	D	P	_	4+	D	3+		4+
68	Treated		D	-	-	3+	D	4+		4+
69	Untreated	-	-	_	D	4+	D	3+		4+
75	Treated	_	D	D	-	_	_	D		3+
228	Inadequate	-	P	P	2+	4+	3+	4+	1	4+
242	Treated	P	P	_	-	4+	D	4+	3 2 1	4+
243	Treated	D	D	P	-	-	-	D	2 1	4+
245	Treated	P	P	P	_	_	_	D	3 2 1	4+
247	Treated	P	D	P	-	3+	D	4+	4 4 4 1-	NT
256	Treated	P	_	P	-	3+	_	2+		3+
286	Inadequate	D	_	P	-	_	-	D		3+
290	Treated	_	-	P	-	D	-	2+		2+
323	Treated	P	NT	P	-		_	2+		3+
324	Treated	P	NT	P	-	-	2+	4+	4 2	4+
				Ne	uro syr	hilis				
34	Untreated	_	P	P	NT	NT	3+	4+		4+
39	Not given	_	P	P	4+	4+	4+	4+	3 1	4+
67	Treated	_	-	_	_	-	-	D		4+
70	Untreated	_	P	P	4+	4+	3+	4+	4 4 4 4 1	4+
86	Treated	P	-	_	_	-	D	3+	2	4+
90	Not given	P	_	P	-	-	_	2+		3+
120	Treated	_	P	P	-	4+	D	4+	1	4+
167	Untreated	P	_	P	-	_	4+	4+	1	4+
226	Treated	_	_	P	D	4+	D	4+		4+
253	Treated	_	_	P	-	D	_	2+		3+
310	Treated	P	P	P	-	3+	2+	4+	3 2 1	4+

TABLE 6

						-				
CASE NO.		EAGLE C.F.	EAGLE M.F.	HINTON	KAHN 8.	KAHN P.	KLINE D.	KLINE E.	KOLMER C.F.	MAZ- ZINI
				Tert	iary sy	philis				
11	Untreated	-	P	P	3+	4+	4+	4+	1	4+
13	Treated	_	P	P	4+	4+	4+	4+	4 4 2 1-	4+
423	Inadequate	-	P	P	2+	4+	3+	4+	2 1	4+
8	Untreated	P	P	-	4+	4+	4+	4+	4 2	4+
				Secon	dary s	yphilis				
307	Treated	-	-	P	-	3+	NT	NT		4+
308	Treated	-	-	P	-	-	-	D		2+
			(Conge	enital s	yphilis	1			
294	Treated	-	P	P	D	4+	D	4+	1	4+
				Prin	ary sy	philis				
231	Treated	D	-	P	_	_	- 1	2+		D
277	Treated	-	-	-	-	-	-	-	4 3 2	-
289	Treated	-	-	P	-	-	-	D		D
300	Treated	D	P	P	-	-	-	-		2+
311	Treated	-	-	P	-	-	-	D		3+
316	Treated	D	P	P	-	-	-	2+		3+

P, positive; -, negative; D, doubtful; NT, no test; M.F., microflocculation; C.F., complement fixation; S., standard; P, presumptive; D, diagnostic; E, exclusion.

TABLE 7
NORMAL PRESUMABLY NONSYPHILITIC INDIVIDUALS (100 CASES)

TECHNICS	TOTAL SPECI- MENS EXAMINED	DOUBTFUL REPORTS	PERCENTAGE OF NEGATIVE REPORTS	NUMBER OF SPECIMENS NOT TESTED
Eagle C.F	91	0	100	9
Eagle M.F		0	100	8
Hinton	99	0	100	1
Kahn S	96	0	100	4
Kahn P	96	0	100	4
Kline D	99	0	100	1
Kline E	99	0	100	1
Kolmer C.F	100	0	100	0
Mazzini	100	1	100	0

the flocculation test for the serodiagnosis of syphilis and that of the tests of the controlling laboratories participating in the 1938 survey is presented in the tables which accompany. Tables 1, 2, and 3 represent the total number of specimens from syphilitic patients divided into six groups. Table 4 shows the results of the combined groups of tables 1, 2, and 3. Tables 5 and 6 present the detailed analysis of every case of syphilis on which one or more of the tests failed to produce a positive reaction. Table 7 gives the specificity of each test in 100 specimens from normal, presumably nonsyphilitic individuals.

SUMMARY

1. A new modification of the slide flocculation test for syphilis is described, based on the use of a beef heart and egg yolk extract, which has been shown to possess a high degree of specificity and sensitivity.

2. The use of lipoids from egg yolk, although weakly antigenic by themselves, permit the employment of minimal quantities of beef heart lipoids without endangering the specificity of the results.

3. The hydrogen ion concentration of the suspension plays an important rôle in the sensitivity of the test; for just as maximum sensitivity of the antigen is obtained at a certain concentration of lipoids, so maximum flocculation is obtained at a definite pH and salt concentration.

4. The antigen suspension can readily be adjusted to different degrees of sensitivity and is easily controlled.

5. Elimination of fluctuation in the sensitivity of every new lot of antigen is accomplished by means of titration.

6. Daily fluctuation in the sensitivity of the antigen suspension is largely eliminated by the use of a buffered saline solution having a constant pH—(6.3-6.4).

7. The test requires a very small amount—0.05 cc.—of serum for its performance; a desirable feature in those cases when blood is obtainable in small quantity only.

8. Certain unsatisfactory features common to some flocculation tests have been eliminated, yielding a simplified technic. 9. A new modification has been introduced for the preparation of the ringed glass slides and the dispensing of the antigen suspension.

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THE SEDIMENTATION RATE AND THE LEUKEMOID REACTION IN METASTATIC TUMORS OF BONE*

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The sedimentation rate of the erythrocytes and the leukemoid reaction as seen in blood smears have both begun to be used extensively in the past few years. Much attention has been paid to the use of these tests singly as an aid in the diagnosis of malignant neoplasms, but so far as we have been able to determine, the correlated value of these two laboratory findings has been neglected.

Cutler^{7, 8} said that the sedimentation phenomenon depends on the amount of cellular destruction going on in the body. Pregnancy is the only physiologic process that is accompanied by rapid sedimentation, and if this be ruled out a rapid rate is otherwise found only in infectious disease and in malignancy. Concerning the value of the sedimentation rate, Bannick, Gregg and Guernsey² said: "It may aid in distinguishing benign from malignant conditions. However, the test must not be relied upon too much in this regard. We have seen patients with cancers of various types and sizes and even with regional and distant metastases in whom the sedimentation rate was perfectly normal. Usually, however, the sedimentation rate is increased in malignant conditions, particularly when the lesion is of the ulcerating type. Therefore, when the physician is trying to

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decide upon whether a given lesion is benign or malignant an increased sedimentation rate is a point in favor of malignancy provided other causes for the increased sedimentation rate can be excluded. However, a normal sedimentation rate under such conditions does not exclude malignancy. Lymphoblastomas and sarcomas usually are associated with an increased sedimentation rate just as are carcinomas."

Golob and Borowsky¹³ stressed the value of this test in gastroenterology. In a group of seven cases of carcinoma of the gastrointestinal tract the sedimentation rates varied from 15 to 37 mm. in four cases in which the patients were males and from 26 to 28 mm. in three cases in which the patients were females. These authors apparently were of the opinion that an increase in the sedimentation rate is of at least some value in distinguishing benign from malignant ulcers.

Gregory¹⁵ found eight instances of malignancy among 1102 patients who were admitted to a state hospital. The average sedimentation rate in these eight cases was 23 mm. in one hour when determined by the Westergren technic. Gram¹⁴, after examining the sedimentation rates of twenty-one patients who had malignant tumors, concluded: "In most cases of malignant tumor the sedimentation is found increased."

Hirsh¹⁶ reported finding a sedimentation rate of 32 mm. in a case in which a fibroid uterus had undergone malignant change. In seven other cases of malignancy the sedimentation rates were more than 28 mm. The Westergren technic was used. Hirsh quoted Schiller²⁷ as follows: "If a malignant growth be removed operatively the sedimentation should return to normal in six weeks if complete removal was obtained. If the rate returns to normal and stays so for six months, a guardedly favorable prognosis may be given. If the rate becomes abnormal, it indicates recurrence or metastasis."

The leukemoid reaction may be defined as a blood picture simulating that of leukemia. This reaction exists in many types of conditions but the diagnosis usually can be established by careful clinical consideration. However, in an occasional case the utmost clinical care and even necropsy will not settle the

question as to the leukemic or leukemoid character of the reaction of the blood. This fact has been clearly demonstrated by Krumbhaar²⁰.

Discounting the various theories as to genesis of blood corpuscles, we may say generally that myeloid, lymphoid, and monocytoid types of leukemia may occur. Similarly leukemoid reactions may occur in each of these three broad types of leu-This reaction of the blood may be attributed to one or two factors which may act singly or in combination. The first of these probably is the toxemia which is caused by severe infections; the second is displacement and stimulation of hematopoietic tissues by tumor tissue. The myeloid type of leukemoid reaction has been noted in acute pneumonia, empyema, puerperal sepsis, tuberculosis⁶ and in numerous other conditions. been produced experimentally by the administration of the salts of mercury¹⁰ and by the administration of tuberculin to tuberculous rabbits. It is well known that a definite eosinophilic leukemoid reaction may occur in the acute phase of trichinosis and may occasionally be present in glandular tuberculosis.

The lymphoid type of leukemoid reaction has been frequently seen in pertussis (Krumbhaar) and in infectious mononucleosis as well as in tuberculosis²², syphilis, and lipoid histiocytosis¹¹. The monocytoid type of leukemoid reaction is occasionally seen in tuberculosis and in syphilis following treatment with neo-arsphenamine¹².

In this work we were primarily interested in the reaction associated with carcinoma, particularly metastatic carcinoma^{5,23}. In neoplastic conditions other than carcinoma, a lymphoid type of blood picture has occasionally been noted in Hodgkin's disease²⁵, lymphosarcoma²⁶, and even in papillary cystadenoma of the ovary³⁰.

A general consideration of the findings in the peripheral blood in cases of metastatic carcinoma might not be amiss. Piney has been an ardent worker in this field and has contributed much to our present knowledge of this subject. According to Piney^{24, 25}, metastatic lesions of bone may produce one or two

types of reaction; the first type is the so-called "pseudopernicious" anemia, and the second is known as pseudoleukemia.

Pseudopernicious anemia may clearly simulate pernicious anemia, but if the patient and the laboratory findings are studied carefully, the diagnosis usually can be made. In this form the color index occasionally may be low. A study of the smear will reveal a granulocytic leukocytosis, while a lymphocytosis usually is noted in pernicious anemia. Anisocytosis and macrocytosis occur in both conditions. However, in pseudopernicious anemia the erythrocytes are polychromatophilic, while in pernicious anemia they are orthochromatic. The large nucleated erythrocytes in pseudopernicious anemia are pale and have a polychromatophilic cytoplasm and cartwheel nucleus; in pernicious anemia the cytoplasm is orthochromatic and the nucleus has a finely reticular cytoplasm.

Humphrey¹⁷ said that if the following criteria are satisfied, a diagnosis of metastatic involvement of the bone marrow can be made incontrovertibly: "1. Absence of definite anemia; a hemoglobin no less than 75 to 80 per cent and an erythrocyte count of at least 4,000,000. 2. Presence of normoblasts in the differential

smears."

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The association of the leukemoid reaction with cancer was established in 1903 by Kast¹⁹ and by Kurpjuweit²¹. Bizzarri³ in 1910 noted a leukemoid reaction in a case of carcinoma of the stomach. Dieballa⁹ and Entz noted myeloid immaturity in a case of spindle cell sarcoma of the pleura with osseous metastasis. In 1923 Piney²⁴ reported myeloid immaturity in carcinoma of the stomach, lungs, and kidney, as well as in a case of Hodgkin's disease in which metastasis had occurred. In 1926 Jaksch¹⁸-Wartenhorst reported finding a leukemoid reaction in a case of carcinoma of the splenic flexure of the colon in which there was metastatic involvement of the spleen. In the same year Seemann and Krasnopolski²⁸ noted a leukemic blood picture in a case of carcinoma of the stomach in which there was metastatic involvement of the bones, lymph nodes, spleen, and mesentery.

The differentiation of leukemia and the leukemoid picture produced by neoplasms may be well-nigh impossible and there is

only one point of much value to the hematologist, that is, the absence, or low percentage, of basophilic cells in the leukemoid reaction.

Piney²⁵ has demonstrated that the metastatic deposits in the bone marrow are carried by the blood and that they therefore are intravascular in position. He said that from the sequence of events it is obvious that the first effect of such deposits will be exerted on the intravascular erythroblasts but at a later stage, when the emboli have grown into the surrounding leukopoietic tissue, there will be a severe irritation of this tissue and consequently a leukemoid reaction.

Our experience has convinced us that an increased sedimentation rate always indicates the presence of disease of some kind but a normal sedimentation rate does not exclude the presence of disease³¹. If a rapid sedimentation rate is found and if infection and pregnancy can be eliminated, one must consider the possibility of a malignant lesion. Metastasis should be considered if the rate is unusually high or if there is anemia that is associated with a marked increase in regeneration of erythrocytes and particularly if a leukemoid reaction is present.

TECHNIC

The sedimentation test. The Westergren technic was used in all examinations. This method consists of adding 4.5 c.c. of freshly drawn venous blood to 0.5 c.c. of 3.8 per cent solution of sodium citrate. The resulting mixture is gently agitated and drawn up into a pipet which is placed in a vertical position in a rack. The pipet, which is 300 mm. in length, is graduated in millimeters for a distance of 200 mm. from the bottom. The rate of sedimentation is read only once, that is, at the end of one hour. Repeated tests were made when they appeared necessary.

Blood smears. The blood was obtained from the lobe of the patient's ear, spread, air dried immediately, and then stained with Wright's stain. Three to five hundred leukocytes were counted in every case and the percentage of the various leukocytes was computed.

MATERIAL

The material used for this study consisted of sixty-two cases of carcinoma. In all but a few of these cases metastatic involvement of bone was proved by roentgenologic examination.

Twenty-seven of the patients were males and thirty-five were females. The site of the primary carcinoma is shown in table 1. The presence of myelocytes, promyelocytes, leukoblasts or myeloblasts was considered satisfactory evidence of myeloid immaturity. The clinical data in these cases are included in tables 2 and 3.

The cases were divided into three groups according to the degree of anemia (table 4). Group 1 included the cases in which the erythrocyte count was more than 4,010,000 per cubic millimeter of blood and cases in which the concentration of hemoglobin was 13.4 gm. or more per 100 c.c. of blood. In selecting

TABLE 1
Site of Primary Tumor in Sixty-two Cases of Metastatic Tumors of Bone

CASES IN WHICH THE PATIENTS WERE	MALES	CASES IN WHICH THE PATIENTS WERE I	EMALE
Site of primary tumor	Cases	Site of primary tumor	Cases
Prostate gland	13	Breast	25
Unknown	8	Unknown	2
Stomach	2	Stomach	3
Kidney	2	Kidney	1
Larynx	1	Uterine cervix	1
Rectum	1	Adrenal gland	1
		Bartholin's gland	1
		Vulva	1
Total	27	Total	35

this group the concentration of hemoglobin was used as the chief criterion if there was a disparity between the erythrocyte count and the concentration of hemoglobin. The second group included cases in which the erythrocyte count was between 3,010-000 and 4,000,000 per cubic millimeter of blood and the concentration of hemoglobin was between 10.1 and 13.3 gm. per 100 c.c. of blood. The third group included all cases in which the erythrocyte count was less than 3,000,000 per cubic millimeter of blood or the concentration of hemoglobin was less than 10 gm. per 100 c.c. of blood.

From this study it would appear that with increasing anemia

there is an increasingly rapid rate of sedimentation out of proportion to that which might be due to anemia alone, and similarly an increase in the probability of finding evidence of myeloid

TABLE 2
CLINICAL DATA IN TWENTY-SEVEN CASES IN WHICH THE PATIENTS WERE MALES

SITE OF PRIMARY TUMOR	AGE OF	BONES IN- VOLVED	SEDIMEN- TATION RATE OF ERYTH- ROCYTES	EXTENT OF MYELOID IMMATURITY	ERYTH- ROCYTES, MILLIONS PER CU. MM. OF BLOOD	HEMO- GLOBIN, GM, PER 100 CC, OF BLOOD	LEUKO- CYTES, PER CU. MM. OF BLOOD
	years		mm,				
Prostate gland	50	4	17	0	4.13	14.5	5,100
Prostate gland	60	3	136	Promyelocyte	2.85	10.1	7,200
Prostate gland	62	3	108	0	3.13	10.7	4,400
Prostate gland	66	2	64	0	3.97	13.3	14,500
Prostate gland	60	2	84	Promyelocyte	3.65	14.5	17,000
Prostate gland	49	2	86	Myelocyte	4.47	15.2	7,000
Prostate gland	56	2	81.5	Myeloblast	4.25	14.9	8,400
Prostate gland	60	2	30	0	4.28	15.2	11,800
Prostate gland	65	2	70	Promyelocyte		15.2	8,700
Prostate gland	71	1	37	0	2.29	9.8	6,500
Prostate gland	61	1	77	0	3.78	12.7	8,200
Prostate gland	76	1	133	0		11.7	
Prostate gland	73	1	141	Promyelocyte	2.49	12.1	6,800
Unknown	77	2	90	Promyelocyte	3.15	7.8	8,400
Unknown	44	4	140	Leukoblast	3.73	12.5	7,500
Unknown	44	2	120	Myelocyte	3.72	10.6	12,600
Unknown	63	2	136	0	4.36	10.4	10,300
Unknown	?	2	47	0	4.35	14.1	14,800
Unknown	61	1	132	Myeloblast	2.78	10	7,400
Unknown	62	1	63	0	4.68	77	11,000
Unknown	54	1	38	Promyelocyte	4.46	14.9	7,400
Stomach	57	?	82	Promyelocyte	4.19	11.4	14,700
Stomach	49	4	154	Myeloblast	2.25	6	9,000
Kidney	59	3	9.5	0	5.06	17.9	8,400
Kidney	46	1	51	0		15.4	,
Larynx	60	1	109.5	0	4.34	13.9	11,300
Rectum	51	1	53	0	5.18	14.2	15,300

immaturity in the blood smear. Contrary to expectation the sedimentation rate does not appear to increase with succeeding involvement of bone although the probabilities of finding evidences of alteration of bone marrow, as reflected in the blood

TABLE 3 CLINICAL DATA IN THIRTY-FIVE CASES IN WHICH THE PATIENTS WERE FEMALES

SITE OF PRIMARY TUMOR	AGE OF	BONES IN- VOLVED	SEDIMEN- TATION RATE OF ERYTH- ROCYTES	EXTENT OF MYELOID IMMATURITY	ERYTH- ROCYTES, MILLIONS PER CU. MM. OF BLOOD	HEMO- GLOBIN, GM. PER 100 CC. OF BLOOD	LEUKO- CYTES, PER CU. MM. OF BLOOD
	years		mm.				
Breast	36	4	147	Promyelocyte	2.33	8.2	7,400
Breast	28	4	114	Promyelocyte	2.75	10.5	20,700
Breast	41	4	142	Myeloblast	3.39	13.3	7,200
Breast	48	4	54	0		13.9	
Breast	55	4	113	Myelocyte	3.97	12.1	3,000
Breast	48	3	123	0	2.27	7.9	6,400
Breast	45	3	154	Myelocyte	4.18	11.8	6,600
Breast	55	3	9.5	0	4.53	16.4	8,000
Breast	41	3	97	0	3.74	12.5	6,400
Breast	56	3	39	0		16.9	,
Breast	47	3	65	Promyelocyte	4.49	14.1	12,600
Breast	58	3	65	Myelocyte	4.15	13.4	6,700
Breast	72	2	60	0	4.39	12.9	10,600
Breast	56	2	60	Promyelocyte	4.03	13.1	5,400
Breast	30	2	84	0	3.88	12	4,600
Breast	48	2	59	0	4.23	15.2	8,000
Breast	48	2	50	0	4.87	14.5	10,100
Breast	51	2	57	0	4.63	13.6	7,000
Breast	59	2	67	0	4.48	13.5	8,300
Breast	61	2	71	0	4.59	13.5	11,100
Breast	42	2	31	0	4.98	13.3	9,400
Breast	40	1	148	Myelocyte	4.41	10.8	7,700
Breast	35	1	31	0	4.36	12.6	5,000
Breast	40	1	98	0	3.99	11.4	11,100
Breast	50	1	43	0	4.67	14.9	10,000
Uterine cervix	43	1	123.5	0	4.16	12.5	9,800
Stomach	40	3	96	Myeloblast	2.98	10.1	7,100
Stomach	53	2	41	0	4.24	10.5	14,300
Stomach	48	2	51	Myeloblast	2.07	5.8	10,700
Unknown	46	1	84	Myelocyte	3.56	11.7	5,600
Unknown	69	î	14	0	3.77		6,300
Adrenal gland	5	4	114	0	2.77	6.12	7,300
Bartholin's gland.	49	2	37	0	3.71	14.7	5,800
Kidney	70	3	45	Promyelocyte	3.89	14.1	9,200
Vulva	62	1	112	0	4.48	11.7	9,200

smear, do seem to increase. The fact that the sedimentation rate does not increase with increasing involvement of bone marrow may be due to maximal microscopic involvement of bone marrow when metastasis first occurs. Extension of these microscopic areas will further irritate the marrow and hence release more immature myeloid cells.

These findings perhaps may be illustrated by a report of two cases. In the first case myeloid immaturity and high sedimentation rate were present before roentgenologic evidence of metastasis was noted. The patient was not anemic.

TABLE 4

CORRELATION OF DEGREE OF ANEMIA, SEDIMENTATION RATE AND MYELOID
IMMATURITY IN SIXTY-TWO CASES OF METASTATIC TUMORS OF BONE

HEMOGLOBIN, GM. PER	CASES	SEDI	MENTATION	MYELOID I	MYELOID IMMATURITY			
100 cc. of blood	Calono	Highest	Lowest	Average	Cases	Per cent		
		mm.	mm.	mm.				
13.4 or more	24	109.5	9.5	53	8	33.3		
10.1 to 13.3	31	154	14	96.8	13	41.9		
10 or less	7	154	37	102.3	5	71.4		

Case 1. A man, aged fifty-six years, came to The Mayo Clinic in May, 1934, complaining of gas, belching and vomiting, which had been present for two months. There had been some burning sensation under the lower portion of the sternum after he had eaten; he had had difficulty of this kind particularly after he had eaten meat or potatoes. He had lost 20 pounds (9 kg.) in the three months before he came to the clinic. He also complained of some pain in the shoulders and over the sacrum and lumbosacral region.

The results of general physical examination were essentially negative except for a slight enlargement of the prostate gland and the question of a nodule in the right lower lobe of the gland. The concentration of hemoglobin was 14.9 gm. per 100 c.c. of blood; there were 4,250,000 erythrocytes and 8,400 leukocytes in each cubic millimeter of blood. The morphologic examination of the smear disclosed an increase in the regeneration of erythrocytes and an occasional normoblast. A differential blood count disclosed 52.4 per cent of neutrophils, 27.4 per cent of lymphocytes, 4.4 per cent of monocytes, 0.8 per cent of eosinophils, 2.0 per cent of basophils, 3.0 per cent of metamyelocytes, 3.0 per cent of myelocytes, 5.6 per cent of promyelocytes, 1.0 per cent of leukoblasts, 0.4 per cent of myeloblasts, and 9 normoblasts among 500 leukocytes.

The diagnosis of leukemia as well as leukemoid reaction had to be considered. The sedimentation rate was 81 mm. at the end of one hour. Roentgenologic examination of the spinal column and thorax did not reveal any evidence of metastasis. There was some difference of opinion as to the nature of the lesion in the prostate gland and it could not be definitely stated that the patient had a carcinoma of the prostate gland.

The patient began to show a spontaneous improvement after a week's stay in the hospital; therefore, the diagnosis of possible carcinoma of the prostate gland and metastasis was questioned. It was felt advisable to send the patient home with instructions to return for reëxamination in three months. The conditions that were considered in the differential diagnosis were carcinoma of the

prostate gland and an early stage of chronic myelogenous leukemia.

On July 19, 1934, the leukocyte count was 16,500 per cubic millimeter and the differential count revealed 50.5 per cent of neutrophils, 29.5 per cent of lymphocytes, 3.5 per cent of monocytes, 0.5 per cent of essinophils, 0.5 per cent of basophils, 3.5 per cent of metamyelocytes, 3.5 per cent of myelocytes, 6.5 per cent of promyelocytes, 1.0 per cent of leukoblasts and 1.0 per cent of myeloblasts. Roentgenologic examination, which had been carried out at the patient's home, revealed widespread metastatic involvement of the skeleton.

Marked anemia, a high sedimentation rate and myeloid immaturity were noted in the following case.

Case 2. The patient was a man aged forty-nine years. Four weeks before he came to The Mayo Clinic the extraction of eight teeth had been followed by severe bleeding of eight hours' duration. The day following the extraction he had bled from the gums for two hours, and three days later he had had mild epistaxis, which had recurred two or three times at intervals of three or four days. Two blood transfusions of 500 c.c. each had been given and some liver extract had been administered intramuscularly. Several days before his

admission severe dyspnea had occurred.

The results of general physical examination were negative except for marked pallor and evidence of loss of weight. Urinalysis did not disclose any abnormality. The concentration of hemoglobin was 6 gm. per 100 c.c. of blood; there were 2,250,000 erythrocytes and 14,700 leukocytes in each cubic millimeter of blood. Examination of a blood smear disclosed myeloid immaturity to the stem cell and an appreciable number of normoblasts. The differential count revealed 45.7 per cent of neutrophils, 26.3 per cent of lymphocytes, 3.0 per cent of monocytes, 1.0 per cent of basophils, 8.3 per cent of eosinophils, 5.7 per cent of metamyelocytes, 2.7 per cent of myelocytes, 2.7 per cent of promyelocytes, 1.3 per cent of leukoblasts, 3.3 per cent of myeloblasts, and 77 normoblasts among 300 leukocytes counted. The sedimentation rate was 154 mm. at the end of one hour.

The patient failed rapidly and died on the fourth day after his admission to the hospital. Necropsy disclosed an extensive carcinoma of the stomach, with metastasis to all organs and to the bone marrow.

In considering the differential diagnosis a question of a chronic myelogenous leukemia was properly raised as the blood picture was compatible with either this condition or a leukemoid reaction. In some cases of chronic myelogenous leukemia bleeding is a prominent symptom, usually as the result of a low platelet count. There was no way in which to distinguish between a true leukemia and a leukemoid reaction by morphologic study. The blood picture in this case was, of course, due to metastatic involvement of the bone marrow.

In our experience the absence of anemia, together with the presence of normoblasts, is not positive proof of metastatic involvement of the bone marrow. This picture may be seen in the early stage of a chronic myelogenous leukemia, particularly if only a casual examination of the blood is made. We have been unable thus far to find any criteria on the basis of which it is always possible to separate definitely the leukemoid reaction of metastatic involvement of bone from an early chronic myelogenous leukemia. The presence of an enlarged spleen may merely serve to complicate the picture. Clinically, splenomegaly would be evidence in favor of a leukemia. In one of the most striking cases of leukemoid reaction which has come to our attention there was marked enlargement of the spleen, which weighed 1250 gm. at necropsy. The increase in the size of the spleen was due to metastatic carcinoma from a lesion in the breast.

SUMMARY

The average sedimentation rate in the entire group of sixtytwo cases was 80.4 mm. in one hour. In thirty-five of these cases there was evidence of myeloid immaturity. The sedimentation rate increased with an increasing degree of anemia, but did not appear to increase with the successive metastatic involvement of bone. The percentage of cases in which myeloid immaturity was present increased both with increasing anemia and with successive involvement of bone.

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THE MYELOID PATTERN IN PERNICIOUS ANEMIA*

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A general study of the hemogram in pernicious anemia was previously reported by two of us¹⁹. Our initial observations were essentially the same as postulated by Schilling¹⁷ except that our counts showed stab forms greatly increased in severe relapse in contrast to Schilling's dictum that only a right myeloid shift is the rule. For this and other reasons, an extensive study of the leukogram in pernicious anemia has been made. The data in this report, therefore, describe in detail the pertinent aspects of the leukocytic patterns in pernicious anemia and correlate them with the well-known clinical stages of the disease. "Typical" hemograms of untreated relapse, early remission, incomplete remission and complete remission are cited with their respective interpretations.

METHOD OF STUDY

The data discussed in this study were derived from approximately 1200 hemograms on 40 patients. A complete hemogram was not made each time a blood count was made, except during the phases of relapse and early remission Blood counts were made daily in the early phases of relapse and several times weekly during the other clinical phases of the study. In order to permit a valid conclusion as to the leukocytic equilibrium a representative hemogram for each of those stages is cited in table 1. While it was impossible to collect all blood specimens at the same time of day on each patient the majority of the counts were made on preparations taken during the early forenoon.

Counting chambers used were the Levy-Neubauer type. Pipettes were approved U. S. Bureau of Standards type. Aqueous acetic acid in 3 per cent solution was used for diluting fluid in making all leukocyte counts. A technical error of plus or minus 1 per cent is allowed in leukocyte counts by the technical procedure reported by one of us (E. M. S.)¹⁸. Normal standards for leukocytes: Total, 6000 to 8000 per cubic millimeter; Differential (Modified Schilling count):

^{*} Received for publication March 21, 1938.

Myelocytes, 0 per cent; Juvenils, 0 per cent; Stab, 4 per cent; Segmented, 63 per cent; Eosinophils, 3 per cent; Basophils, 1 per cent; Lymphocytes, 23 per cent and Monocytes, 6 per cent.

A "margin-free" film is made by taking blood from a finger-tip which has been prepared by rubbing briskly with a small sponge moistened with ether. A standard lancet (7 x 3 mm.) is inserted to a depth of 1.5 to 2 millimeters into the finger-tip. The first drop exuding is taken up on gauze, the second drop is picked up with the edge of a cover-slip (18 x 18 mm.), applied at an angle of 45° to the end of a cool microslide on the operator's right, the blood drop is allowed to spread the width of the cover-slip, which is then pushed toward the left with an even, rapid stroke. The preparation is dried at room temperature.

Films were stained with May-Grünwald and Giemsa stains as follows: The margin-free blood film is covered with 0.3 per cent alcoholic solution of May-Grünwald dye and allowed to stand for several minutes. An equal amount of buffer solution at a pH 6.8 is added, and after standing one minute the mixture is drained off. While the preparation is still wet cover with an aqueous solution of 0.1 per cent Giemsa dye. After standing five minutes the slide is rinsed and air dried.*

The nucleus of the neutrophil should be clear and bluish in color—not pale nor purple. The nucleus should be sharply demarcated when the stain is properly used. The cytoplasm should show granules, vacuoles and all detail in sharp relief.

Four hundred leukocytes are counted for the differentiation. Enumeration of all cells is based on an Arneth-Schilling classification. It is important to note that estimation of the lobes of the neutrophilic nucleus is based on the number of segments distinctly separated by filaments.

Typical Hemogram in Relapse. The typical quantitative and qualitative aspects of a hemogram in relapse of pernicious anemia can be described briefly as a paradoxical blood picture, since in a majority of uncomplicated, untreated relapsed cases it shows the following characteristics:

(a) Leukopenia. The total leukocyte count is usually below 5500 per cubic millimeter. In Chart 1 are given the total white and red blood cell counts on 150 untreated cases of pernicious anemia in relapse. Of this number 129, or 84.2 per cent, showed eythrocyte levels at or below 2,500,000 per cubic millimeter. Inasmuch as this level of erythrocytes is regarded as denoting

^{*} Technic reported in detail by Schleicher and Sharp: Rapid Methods for Preparing and Staining Bone Marrow, J. Lab. Clin. Med., 22: 949 (June) 1937.

severe relapse, only total leukocyte counts appearing in the 129 cases will be considered. It will be seen in Chart 1, therefore, that 110, or 85.4 per cent, of the 129 cases showed a total leukocyte count at or below 5500 per cubic millimeter. Pretreatment leukocyte counts in untreated cases of relapsed pernicious anemia

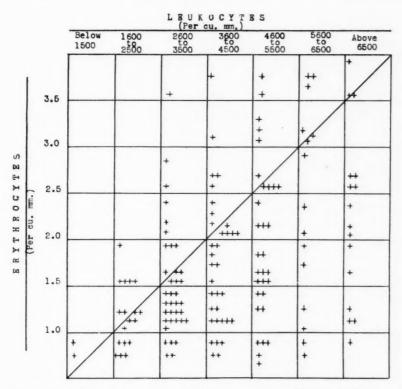


Chart 1. Initial erythrocyte and leukocyte counts on 150 untreated cases of pernicious anemia showing the leukopenic trend during the phase of relapse.

tend to low levels. While the downward trend of the leukocytes is comparable to that of the erythrocytes, there is not demonstrable, in our data at least, any directly proportionate relationship between the total number of leukocytes and the erythrocyte concentration in the untreated phase of relapse.

(b) Neutropenia. The neutrophil series may not exceed 40

to 50 per cent of the differential count during the anemic stage of pernicious anemia. A neutrophil percentage of 45 often will be found. Of this number (45 per cent) the majority consists of the segmented forms, which show a more or less pyknotic nucleus and numerous cytoplasmic granules of various sizes. The multi-

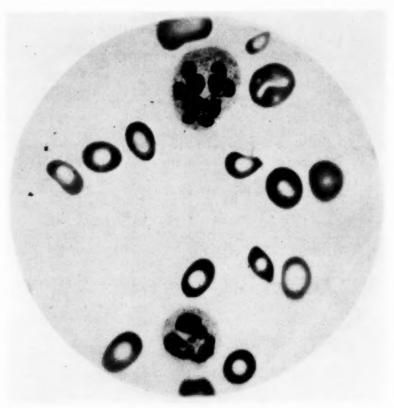


Fig. 1. Hypersegmented neutrophil of severely relapsed pernicious anemia. $\times\,1200,$

lobed neutrophil, regarded by Arneth as indicative of cellular hypermaturity, is usually found. Cells of this type showing 6 to 8 lobes are common in severe relapse and less frequently are found those having 10 to 12 nuclear lobes. Figure 1 is a photomicrograph of an oversegmented form showing 10 nuclear segments.

In addition to neutropenia, the ratio between segmented and stab forms is unbalanced in the anemia phase of pernicious anemia, as compared with the normal distribution. A reduction in the number of segmented cells with a greatly increased number of stab neutrophils is the rule. The percentage of stab cells may show an increase three times above the normal.

Accompanying the imbalance between the normally present definitive forms of the neutrophil, primitive cells of the series appear in the pattern in practically all cases of severe relapse. The immature forms, juvenils and myelocytes, may constitute 2 to 10 per cent of the neutrophil group. While the left shift may include myelocytes in only 10 to 15 per cent of the pretreatment leukocyte patterns, less than 10 per cent of the hemograms failed to manifest an appreciable number of juvenil forms.

Summarizing, then, neutropenia associated with a decreased number of segmented neutrophils, an increased number of stabs, the appearance of juvenils frequently and myelocytes occasionally, constitute the quantitative changes in the neutrophil series in untreated relapsed cases of Addison's anemia. These quantitative features together with the abnormal cellular structure described above represent in the hemogram a left shift of a degenerative-regenerative type. Emphasis on the retrogressive character of the shift is essential since the morphologic defects in the protoplasmic and nuclear structures of the neutrophil series denote degenerative influences. There are practical difficulties, however, in arriving at conclusions about cytologic pathology. Uniform staining technic is mandatory and experience is essential in order to make dependable interpretations.

In severe relapse of pernicious anemia, the neutrophils display a classical picture of degeneration. Primarily, the nuclear bodies vary in size, being consistently smaller than normal in relation to the cellular diameter. The nucleus is pyknotic. The staining reaction of the neutrophil is frequently abnormal, also. There is a lack of uniformity in cytoplasmic color due to various degrees of degeneration of the protoplasm, vacuolation, and the presence of irregular sized granules either isolated or clumped together.

Increased neutrophilic fragility is demonstrable in the fixed film,

an increased number of crushed and disintegrated forms being found.

The foregoing cytologic changes constitute the usual degenerative aspects of the neutrophils in the typical pretreatment leukocyte picture in relapse of pernicious anemia.

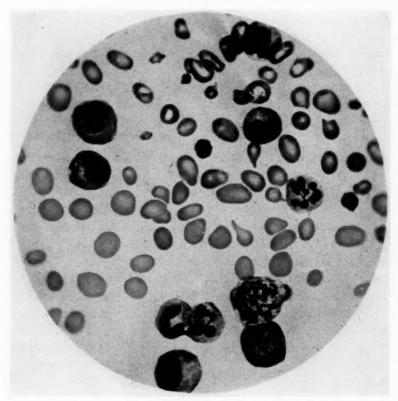


Fig. 2. Pattern of bone marrow from a severely relapsed case of pernicious anemia showing the myeloid cell series including a hypersegmented neutrophil. \times 900.

At this point it is desirable, also, to remark that these degenerative changes are found in the immature neutrophils as well as in the stab and segmented forms. The concept that primitive myeloid cells should appear in the peripheral blood in a normal histologic configuration is erroneous. Figures 2 and 3 show

clearly the reason that all neutrophil types encountered in the fixed film made from peripheral blood during relapse would be expected to manifest degenerative injuries. Figure 2 is a photomicrograph of sternal bone marrow taken from a severely relapsed

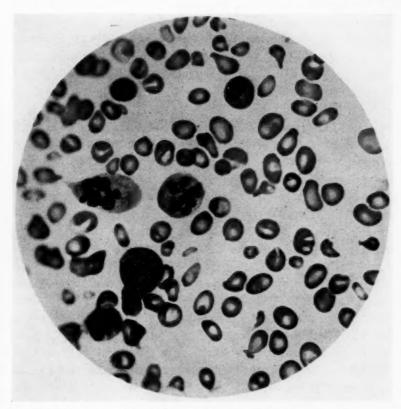


Fig. 3. Pattern of peripheral blood taken from the patient at the same time as the bone marrow biopsy represented in figure 2. The myeloid series of cells, including a hypersegmented neutrophil, is comparable to that of the bone marrow. \times 900.

untreated case of Addison's anemia. Figure 3 represents a fixed film of peripheral blood taken from the same patient at the time the bone marrow was obtained. The morphologic features of the neutrophils in the bone marrow preparation are indistinguishable from those in the fixed preparation of the peripheral blood. The

myeloid series in the bone marrow and peripheral blood show similar degenerative changes. The inference is that the cytologic degeneration is an expression of defective neutrophilic leukopoiesis, resembling that frequently seen in myelotoxemia.

(c) Lymphocytosis. An appreciable relative increase in the percentage of lymphocytes is found in the hemogram during the untreated phase of relapse. The percentage of lymphocytes may be as great as 60, two or three times the normal number, as in patients Nos. 10 Hu, 27 Ry and 32 Ch, table 1, the degree of lymphocytosis depending on the neutropenic state.

(d) Monocytopenia. Since the trialistic theory of blood formation imputes a reticulo-endothelial origin to this type of leukocyte, it is considered separately. In the normal adult blood pattern the monocytes account for about 6 per cent of the leukocytes. In severe relapse the monocytes are usually decreased in number or entirely absent. The monocyte has no particular significance in the hemogram of typical pernicious anemia. In view of the frequent toxemic complications in pernicious anemia patients, however, the behavior of the monocyte during the various clinical phases of the disorder should be given further study.

The Hemogram in Remission. Erythropoiesis resulting from induced remission of Addison's anemia follows a fairly definite and constant course. These changes may be defined as being principally a conversion of an immature red blood cell pattern into one of maturity. Paradoxically, the significant alteration in the leukocytic pattern during remission is the converse of that seen in the erythropoietic system. The hypersegmented neutrophil of relapse may or may not disappear in early remission but an appreciable increase in immature neutrophils (hyperneocytosis) is the rule. The left nuclear shift may even include myelocytes in this phase. It will be seen in table 1 (Column B) that the myeloid shift becomes essentially regenerative in character early in remission. That is to say, in most instances neutrophilia has supplanted a neutropenia, and immature forms (juvenils and myelocytes) if present in the pretreatment blood pattern, show an increase in percentage, or if absent from the pretreatment hemogram will appear when erythropoiesis is initiated. Simul-

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taneously with these leukopoietic phenomena degenerative processes in both cytoplasm and nuclei disappear.

Other minor morphologic alterations in the leukocytic picture accompany the regenerative left shift. Among these changes are the return of the lymphocytes to a normal percent relationship and a sharp increase in the number of monocytes. Finally, the numerical equilibrium is restored coincidently with complete erythropoietic remission.

Noteworthy among the hemologic lineaments of leukocyte behavior during induced remission of pernicious anemia is the persistence of a primitive nuclear deviation to the left. As was asserted above, the myeloid pattern changes from a definitive to a primitive form as remission progresses. In table 1 it will be seen that, scarcely without exception, the juvenils persist in a greatly increased percentage and stab forms generally continue to make up 10 to 20 per cent of the neutrophil series. These characteristics of remission are so constant that they warrant special discussion.

In a previous section were described the typical hemograms of relapse and remission in pernicious anemia. Atypical patterns are encountered and deserve special consideration.

In Chart 2 is cited an atypical hemogram of relapse in patient McD, female, 69 years of age, who had infected hemorrhoids. There was also present cardiac decompensation. The entire picture was one of severe hemopoietic depression. Although the infection was not sufficiently dynamic to cause a hyperleukocytosis and a neutrophilia, yet extreme degeneration of the stabs and a marked shift to the left were indicative of toxemia.

Hyperleukocytosis may ensue in any clinical phase of Addison's anemia as a result of invading noxa. In most instances an acute infection will produce a neutrophilia, which is accompanied by an extreme degenerative shift to the left. The neutrophilia under these conditions usually consists of an increased number of stabs and appearance of juvenil and myelocytic forms. The multilobed or hypersegmented neutrophil is seldom found in the hemogram of relapse associated with severe toxemia.

TABLE 1

Representative Hemograms of Myeloid Reaction on 40 Patients at Three Different Red Blood Cell LEVELS DURING INDUCED REMISSION OF PERNICIOUS ANEMIA

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4	.vM		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEMOGRAM—COMPLETE REMISSION	Hb.	per	110	100	102	96	110	100	103	102	108	100	86	100	100	108	100	109	100	110	108	105	100
HE	ВВС	mil.	15.7	25.0	35.1	44.8	55.1	65.0	75.2	85.1	95.5	5.0	14.9	25.0	8.48	15.1	55.0	35.0	74.9	35.0	5.1	0.2	10
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GRA	.vM		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEMOGRAM—INCOMPLETE REMISSION	HP.	per	74	92	11	06	100	86	08	110	92	102	92	06	84	100	28	66	92	104	94	86	90
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REL	,38		11	14	8	15	25	11	20	17	16	10	12	13	6	6	12	32		12		12	
EA LD	Juv.		3	0	4	4	C	4	-	0	50	9	CA	က	-	2	4	5	5	3	7	0	_
MI	.vM		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
HEMOGRAM—EARLY REMISSION MILD RELAPSE	HP'	per	62	74	62	29	74	74	20	8	80	65	80	28	80	74	84	78	62	20	28	82	OC.
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NEN	Ba.		0	0	7	N	-	N	-	-	-	0	П	0	-	0	-	0	-	-	0	-	C
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LAP	Seg		46	51	32	38	46	21	34	42	39	24	47	32	42	09	44	49	35	53	40	40	40
GEVERE RELAPSE	.38		1	20	16	13	12	32	14	6	14	10	12	14	14	0	28	12	16	14	17	17	14
ERE	Juv.		2	-	1	CI	0	0	-	2	2	10	2	1	N	0	0	3	5	4	73	2	
EV	My.		0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0
HEMOG	Hb.	per	62	65	45	64	20	65	49	42	48	38	62	22	53	35	28	62	42	80	38	53	46
H	BBC	mil.	00	2.2	.5	2.4	6.0	00	1.7	1.3	2.1	9.1	2.2	1.1	1.7	1.1	0.0	6.1	1.5	8.2	1.4	2.1	1 8
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5	33	3	5	33	3	00	3	5	3	01	2	3	3	5	2	3	33	3
2 22	2 23	424	3 25	3 26	0 27	0.28	129	230	031	032	3 33	234	5 35	036	337	238	2 39	2 40
32	34	3	53	38		34	48	53	26	99		46	24	47		42		44
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2	_	11	31	4	-	92	2	4		67	_	0	-	0	2	1	0	
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Not only are the quantitative features of a hemogram in relapse of pernicious anemia rendered atypical by the behavior of the myeloid series in response to toxemia but cytoplasmic and nuclear degenerative characteristics, notable in relapse, persist until the associated disorder is eliminated or ameliorated. A degenerative-regenerative shift, therefore, manifests itself in pretreatment observations and continues even though erythropoiesis may ensue at a satisfactory rate.

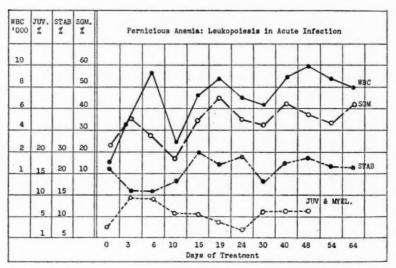


Chart 2. Hemogram of severely relapsed pernicious anemia showing abnormal leukopoiesis due to infected hemorrhoids.

In summary, leukocytosis, neutrophilia, a degenerative-regenerative myeloid reaction and a quantitative left shift in the neutophil group (and in most instances a continued monocytopenia and eosinopenia) should be interpreted as evidence of a pathologic process complicating the anemia. It can be said, moreover, that if associated disorders are not eliminated or ameliorated a satisfactory erythropoietic response should not be expected.

In order to clarify the discussion of the hemogram found in pernicious anemia a tabulation (table 2) of the salient qualitative and quantitative features of the typical hemograms of severe relapse and complete remission described above are contrasted with the atypical hemograms in the same clinical phases. Since the leukocytic changes of early remission and incomplete remission are fully described elsewhere and are merely arbitrary gradations in the course of a well-known hemopoietic process between the extreme of severe relapse and complete remission they are omitted.

TABLE 2
Comparison of Hemograms in Severe Relapse and Complete Remission

LEUKOCYTE	SEVERE	RELAPSE	COMPLETE REMISSION							
ELEMENTS	Typical	Atypical	Typical	Atypical						
Total W.B.C	1000-6500	Above 6500	6000-8000	Leukopenia or leu- kocytosis						
Neutrophils	Neutropenia	Normal or neutro- philia	Number normal (58-66 per cent)	Neutropenia o						
(a) Segmented	Number decreased, right shift	Absence of right shift	Number normal (63 per cent)	Numberincreased						
(b) Stabs	Degenerative types in in- creased number	Increased or num- ber normal	Normal type and increased	Normal or in- creased						
(c) Juvenils	Frequently pres- ent (1-5 per cent)	Greater than 5 per cent	Occasional	Above 2 per cent						
Myelocytes	Rare (1-2 per cent)	Greater than 2 per cent	Absent	Present						
Lymphocytes	Lymphocytosis	Normal number or lymphocytopenia	Number normal	Lymphocytopenia						
Eosinophils	1 to 4 per cent	Absent or eosino- philia	Number normal	Variable						
Basophils	Absent, normal or increased	Absent	Number normal	Variable						
Monocytes	Decreased to absent	Normal number or monocytosis	Normal number or decreased	Absent or in- creased						

- 1. A degenerative-regenerative left myeloid shift is typical of relapse and occurs coincidently with a nuclear shift to the right.
- 2. A regenerative left myeloid shift ensues concurrently with erythropoiesis, while degenerative cytologic changes tend to disappear.
- 3. The right nuclear shift and neutropenia of relapse is replaced by neutrophilia and normal nuclear segmentation coincidently with reticulocytosis and the initiation of erythrocytic maturation.

4. A definite regenerative left myeloid shift persists after remission of the anemia of pernicious anemia is complete.

DISCUSSION

One of the most popular uses of the Arneth-Schilling hemogram is in diagnosis of infection and toxemia. Failure to derive information from the hemogram when it is applied to a study of infection or any other entity is largely due to unfamiliarity with the salient cytologic features of leukopoiesis under normal conditions. By the same token, when the hemogram is to be used in a study of pernicious anemia, not only the blood pattern in health must be known but a comprehensive concept of the typical leukocyte changes in the various clinical phases of uncomplicated Addison's anemia must be recognized. While this precept would appear to be academic it is not infrequently disregarded.

The following discussion will be offered, therefore, with the injunction to remember that reference to a "typical" hemogram does not imply that the blood picture is that found in health. When referring to a typical hemogram in any one of the four phases of pernicious anemia, it is that picture described above as characteristic of that phase of pernicious anemia unmodified by any other detectable disease entity.

There is a paucity of expression from American hemopathologists on the leukocytic pattern in pernicious anemia and still less discussion of the application of the complete hemogram to a study of this anemic state.

Arneth,¹ Cooke,⁵ Naegeli,¹⁴ Piney¹⁵ and Schilling¹⁻ are among those who have discussed at some length the hypersegmentation of the neutrophil in Addison's anemia. Briggs is credited by Heck and Watkins⁵ with being the first observer in this country to report on an increase in the number of nuclear lobes in the neutrophil in the relapsed phase of pernicious anemia. In a similar study Heck and Watkins⁵ found increased nuclear lobulation of the neutrophil was the rule in 50 cases of Addisonian anemia, although a left nuclear deviation without hypersegmentation occurred also. Further, they could not establish any correlation between the degree of anemia, number of leukocytes and increased

nuclear lobulation of the segmented neutrophils. In our experience, six or more nuclear lobes can be found in a majority of severely relapsed cases of pernicious anemia. It is our impression, however, that this bizarre myeloid form may not indicate hypermaturity, as Arneth² and Schilling¹⁷ have asserted, but immaturity. We are in accord with Jones¹¹ in that hypersegmentation of the neutrophil might be interpreted as the effect of altered function of the bone marrow on the primitive myeloid cell.

The presence in relapse and persistence throughout erythropoietic maturation of a left myeloid shift invites speculation. Doan⁶ postulates that mild reactions affecting the myeloid function of the bone marrow may be detected by an increase in percentage of neutrophils having only two lobes, or no lobing of the nucleus. The same observer elaborates the dictum by asserting that an extension of the shift to the left and its degree may be ascertained by partitioning the myelocytes according to their cytoplasmic structures from which myelopoiesis may be estimated quite accurately.

Heck⁷ has reviewed several reports from various sources relative to myeloid immaturity in pernicious anemia. He cited Naegeli's assertion that myelocytes are found in a majority of cases of pernicious anemia. Zadek also is quoted by Heck to the effect that myelocytes are more frequently present than absent. Heck⁷ reported his own observation on 65 cases of pernicious anemia and postulated that immaturity occurs primarily in cases in which erythrocytes are below 2,000,000. Heck counted 500 cells and classified them according to Pappenheim's terminology.

Brugsh and Naegelbach have recently reported the appearance of myeloblasts, myelocytes and juvenil neutrophils in the peripheral blood of two cases of pernicious anemia immediately following the administration of liver. These observers found a restoration of myeloid imbalance as erythropoiesis progressed. Jaffé¹⁰ found 5 per cent myeloblasts, 13 per cent premyelocytes and 6 per cent metamyelocytes in a case of pernicious anemia before liver treatment. In Jaffé's case the primitive leukocytes disappeared within a few weeks after treatment with liver. This

observation of Jaffé is unique and contrary to our observations inasmuch as in the majority of our cases the juvenils persisted in the leukocyte pattern until the red blood elements reached normal.

Hoffman⁹ has recently reported a study of the leukocytes in 57 cases of Addison-Biermer's anemia. He found that the total leukocyte counts varied between 2000 and 3500 per cubic millimeter in severe relapse, exceeding normal during reticulocytosis, but as a rule, returned shortly to a normal level and remained so throughout remission. Lymphocytosis was present in a majority of relapsed cases, according to Hoffman, and monocytopenia was the rule. In complete remission this observer found monocytopenia very frequently. The eosinophils were reduced to absent in Hoffman's relapsed cases but were increased beyond normal following liver treatment, while basophils showed about the same tendency in relapse, although they did not exceed normal during remission. Hoffman found a left myeloid shift, including myelocytes as well as a right shift in relapse but the phenomena seldom occurred together. He reported one case as showing 18 per cent myelocytes during the reticulocyte phase of remission. The stab forms in Hoffman's series were abnormally high also, fluctuated in relapse and returned to a normal percentage after remission was complete.

In the series of cases covered in this report and from less complete data on myeloid reaction in about 150 additional cases, there is substantial evidence to show that complete erythropoietic recovery is accompanied by a regenerative left myeloid shift. It is evident also from numerical data in table 1 that this is due to an increased percentage of stab forms. In the event, however, that an extreme left degenerative shift continues in full remission it is our experience that a latent toxic process exists. While the percentage proportion between the immature myeloid cells varies in patients, morphologically and numerically the general pattern of the hemogram remains uniform for the individual throughout remission. Slight changes in the shift occur from day to day, but any sudden qualitative or quantitative myeloid deviation which persists should invite investigation of the patient for signs of

intercurrent disease. It is recognized, of course, that abnormal myelogenesis is a common response to toxemia. With the exception of the leukopenia found in influenza, typhoid fever, tuberculosis and malaria, the classical leukocytic pattern in toxemia is neutrophilia and hyperleukocytosis. Some prognostic value, therefore, attaches to this pattern in Addison's anemia, since the anemia will usually prove aregenerative as long as toxemia persists.

In pernicious anemia, a degenerative-regenerative left shift accompanying neutrophilic hypersegmentation, neutropenia and leukopenia, constitute a paradoxical myelopoietic phenomenon. Neutropenia of severe relapse may be regarded as being due to inhibitory influences exerted on the bone marrow. Its concomitant hyperneocytosis, manifested by the constant presence of a substantial increase in the percentage of stab forms accompanied by primitive neutrophils, however, favors another interpretation. The mechanism producing the left shift in relapsed cases may be explained by a disturbed neutrophilic maturation and proliferation. The persistence of the hypersegmented neutrophil in relapse would be consistent with this assumption. During the course of normal myelopoiesis the hypersegmented neutrophil is never found. The cell is found only in states of hypoleukopoiesis. Its presence might be attributed to an accelerated leukopoietic process by which a few primitive myeloid cells escape the intermediate phases of maturation.

In a consideration of our data on the myelocytic pattern of severely relapsed Addison's anemia, the relationship of myelogenesis to blood-maturing substances should be mentioned. Is myeloid immaturity of pernicious anemia due to a lack of the same blood-forming factor known to induce erythropoiesis? It would seem that this conception is at variance with current concepts. At the same time, an analysis of the hemopoietic phenomena incident to induced remission attests the rationale of this interpretation. Scarcely without exception the initial myelopoietic response to adequate treatment of pernicious anemia with antipernicious anemia preparations of stomach and liver is an accession of neutrophilia and an increase in the total

count of leukocytes (table 1). In addition to reports on this point cited in previous paragraphs others have commented on this hemopoietic reaction. Murphy¹³ reported neutrophilia in pernicious anemia ensuing within a few hours after injections of liver extract. Subsequently Powers, Murphy and Humphreys¹⁶ gave normal human subjects liver extract parenterally and induced thereby definite leukocytosis. Meyer, Middleton and Thewlis¹² detected leukocytosis in cases of chronic leukopenia, Banti's disease and in splenectomized persons three or five days after liver extract solutions were given intravenously. It would appear that the myeloid depression and immaturity in relapse of Addison's anemia may be attributable to a deficiency, an absence or non-utilization of the same or similar biologic factors responsible for initiating and promoting erythropoiesis.

The myeloid response does not seem to be modified by the type of antipernicious anemia preparation used to induce remission, except in minor respects. Leukopoiesis is accelerated by parenteral liver extract in contrast to a slower leukocytic change following the oral use of liver, liver extract and stomach. As is well known, eosinophilia accompanies hemopoietic activity when induced by liver, but does not occur following the ingestion of Ventriculin (N.N.R.). It can be said, therefore, that the typical hemograms ascribed to the various clinical phases of pernicious anemia cannot be substantially altered by the form of therapy. This is supported by hemologic data which were derived from blood studies made on patients treated with both parenteral and oral liver preparations and Ventriculin.

SUMMARY

- 1. The typical hemogram of pernicious anemia in relapse shows leukopenia in a majority of cases, characterized by a neutropenia.
- 2. No constant quantitative relationship exists between the respective concentrations of the leukocytes and the erythrocytes in the phase of relapse.
- 3. Bone marrow and peripheral blood patterns taken during pretreatment periods of severely relapsed pernicious anemia

show many immature myeloid forms which is analogous to the primitive erythrocytic pattern found during the same phase.

4. Paradoxically, a right nuclear shift is found in a majority of cases in severe relapse accompanied by a degenerative-regen-

erative left nuclear shift which may include myelocytes.

5. The hypersegmented neutrophil (macropolycyte of Cooke) is found in numerous cases of severe relapse of pernicious anemia. Recently its presence has been attributed to altered bone marrow function rather than hypermaturity. In this report we have suggested that an accelerated leukopoietic process may account for the oversegmentation since the cell is found coincidently in the bone marrow and peripheral blood.

6. Paradoxically, also, myeloid hyperneocytosis accompanies erythropoietic maturation induced by therapy at which time the

hypersegmented neutrophil disappears from the picture.

7. Myeloid immaturity does not disappear usually until the red blood elements are restored to a normal level and balance. Even when this point in recovery is reached, metamyelocytes frequently and an abnormal number of stab forms scarcely without exception can be found in the leukocyte pattern. Both the qualitative and quantitative features of the regenerative shift to the left may continue indefinitely. The degree of shift varies within physiologic limits.

8. In the absence of complications the leukocytic pattern in pernicious anemia is constant, although slight individual variations are manifested by each case of pernicious anemia which persist relatively through all clinical phases of the disease.

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THE DIAGNOSIS OF TRICHINOSIS BY THE DIGESTION METHOD*

AN ADDITIONAL AID TO DIAGNOSIS

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During the past five years reports from widely separated parts of the United States have reacquainted us with the wide-spread prevalence of human infestation with Trichinella spiralis^{1,2,3,4,5}.

6.7.8. Published data of the incidence as determined from examination of post-mortem material ranges from a 3.5 per cent in New Orleans⁷ to as high as 27.6 per cent in Boston¹. An evaluation by Hall in 1937 of the published data indicates that the probable incidence of infestation in the American people is greater than 17 per cent⁵. Scheifly in 1938 places the figure at 20 per cent⁸.

Despite these data we are still without information of the morbidity of this disease. Because trichinosis is considered as a rare disease it is not commonly thought of in differential diagnosis, and undoubtedly many cases escape diagnosis. While it is probably not true that 20 per cent of our population at some time have trichinosis clinically, the experience in epidemics makes it evident that the disease is much more common than present morbidity or mortality statistics indicate. The common history of most epidemics is for the earlier cases to be diagnosed as influenza, typhoid, grippe, or some other such condition. Isolated and sporadic cases probably also receive such erroneous diagnoses. Doubtless the true infestation rate lies somewhere

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between the clinically reported rare incidence of the disease and the 20 per cent⁸ infestation indicated from the examination of bodies at post-mortem and in dissection rooms. Since the incidence of the disease is much greater than formerly thought, any simple laboratory procedure which may be of aid in the diagnosis of trichinosis is to be welcomed by pathologists.

Excepting the rare finding of trichina larvae in the circulating blood, spinal fluid and other body products, there are at present five procedures at the disposal of the laboratory as aids in the diagnosis of trichinosis, namely: the differential white blood cell count, the intradermal test, the histological study of muscle biopsies, the press preparation study of muscle biopsies, and the examination of muscle biopsies by the digestion method. The latter three may also be applied to suspected meat products. It is the use of the digestion method that we particularly wish to advocate.

A high percentage of eosinophiles in the circulating blood is strongly suggestive of trichinosis, and when coupled with presumptive physical findings an elevated eosinophile count may perhaps be considered diagnostic of trichinosis. However, in the absence of such clear cut findings the eosinophile count alone cannot be relied upon to confirm the diagnosis since eosinophilia of varying degrees occurs in such diverse conditions as allergy and the erythroblastic anemia of Cooley, as well as in parasitic infections generally.

The intradermal test as developed by Bachman¹⁰ and McCoy and others¹¹, when positive is strongly presumptive for trichinosis, especially in clinically suspicious cases. Perhaps this test is at its best about 90 per cent accurate. Since most of the people harboring trichina in their bodies also react positively to the intradermal test, it is possible that a patient may give a positive skin reaction when he is not at the time suffering from the clinical disease trichinosis. For these and other reasons the intradermal test, while helpful, is not diagnostic⁹.

Histological sections of muscle removed for biopsy when containing trichina are diagnostic of present or past infection with trichina. However, the trichina, unless very numerous, may be

missed when but few sections are made, and the serial sectioning of biopsies involves a great deal of work. We agree that "the final proof of the diagnosis must rest on the demonstration of the presence or absence of the trichinella" 12. It is true that myositis alone, especially if eosinophilic in character, is considered by most pathologists to be diagnostic of trichinosis. While there are probably no conditions of myositis that can be confused with trichinosis in which a good history will not reveal the true etiology, nevertheless the presence of myositis alone cannot be considered as certainly indicative of clinical trichinosis. Moreover, the absence of myositis does not rule out the presence of trichina since the reaction is transitory, and may be minimal in any given case.

Very few laboratories utilize the press method. In our hands¹ and in those of others¹,³,¹³ this method has not been very satisfactory nor reliable, and we feel it is considerably less accurate than the digestion method. It is true that Pote¹⁴, by means of meticulous technique, has found the press method as accurate as the digestion method. It is our opinion that the exceeding care used by Pote is not adaptable as a routine laboratory procedure, however useful it may be in investigative work. Nevertheless the press method is useful for a quick preliminary observation and it has the merit that the material used for the press observations can later be subjected to the digestion treatment.

The digestion method has been widely used in experimental studies for the isolation of trichina larvae from muscle but so far as we know this method has not been used by pathologists for diagnostic purposes. Since this procedure is simple, accurate, and rapid it should be used on every biopsy from patients suspected of having trichinosis, as well as for the examination of meat products under suspicion. A small segment of the material may properly be used for histological section (to determine the presence or absence of myositis) while the remainder of the specimen is digested. Since the digestion method concentrates all the trichina larvae present in the entire specimen none present are missed, as may be by the section method, unless suitable serial sections are made of the entire material submitted from biopsy.

METHOD

The principle of the digestion method consists of the separation of the dead muscle from the living trichina larvae by digestion of the muscle with artificial gastric juice, the living larvae not being affected by the gastric juice. The digestate is then cleared (if necessary) and examined directly for the presence of the larvae.

The procedure is as follows: Artificial gastric juice is prepared following the directions found in any of the standard biochemistry texts and as used by us consists of a tap water solution containing 1.0 per cent hydrochloric acid and 2.0 per cent powdered pepsin. The solution may be made up fresh or may be kept fairly well in the ice box. After two or three weeks in the ice box a mold may grow, but this apparently does not affect the potency of the solution, since

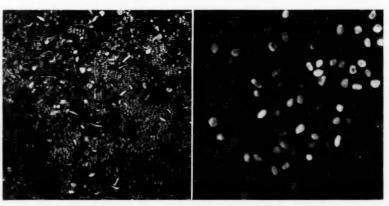


Fig. 1. Isolated living Trichina larvae (× 14). (Reduce 2/3)

Fig. 2. Isolated Trichina cysts (× 14). (Reduce $\frac{2}{3}$)

we have found its action satisfactory after 6 weeks storage. The biopsy material if larger than 5 mm. in diameter is cut into pieces of about this size and incubated in a small beaker (the bottom of which is marked into quadrants) in 8 to 10 times its volume of artificial gastric juice at 37°C. for the time necessary to complete the digestion of the muscle—usually 6 to 12 hours. The living larvae will withstand digestion for 72 hours and longer so none are lost if the material is examined 24 hours or more later. Excess quantities of gastric juice do not harm the procedure.

When the muscle is completely digested the digestate is cleared for observation by withdrawing the supernatant fluid with careful suction. This may be carried out with a vacuum line or by means of a hand syringe. Usually when but 5 or 6 mm. of fluid remains in the beaker, the bottom can be sufficiently well seen over a black background for examination. If not sufficiently clear,

warm tap water is added and the flotsam is removed by suction as above, after allowing 4 or 5 minutes for the trichina larvae again to sink to the bottom. (Water from the warm tap usually emerges milky in color, due to the lesser capacity of warmed water to contain dissolved air. These minute bubbles serve admirably to float debris to the top of the beaker, while the living larvae and heavier cysts gradually settle.) After this procedure one can always see the bottom of the container with sufficient clarity for examination. The sediment is then studied with a binocular dissecting type microscope at about 20 diameters magnification. Strong light from the side facilitates this. The living larvae when present are easily seen and are characteristic. Nothing else mimics them. (See fig. 1.)

Encysted trichina too are characteristic (see fig. 2) and when found indicate an old infection of perhaps years or at least several months standing.*

EXPERIMENTAL DATA

To determine the relative effectiveness of the examination of trichinous tissue by the digestion method, a modified serial section method, and the press method, modified serial sections were made from 73 human diaphragms known to contain trichina larvae, dropping 60 mu between each section saved, and retaining ten or more sections from each specimen, and 29 trichina infected diaphragms were examined with the press method by pressing 2 fields each 4 x 6 cm. and examining them directly with a binocular microscope at 30 diameters magnification. These results are shown in table 1.

It is interesting to note that of the total 402 cases from which in this series was drawn 18.6 per cent were positive for trichinosis by the digestion method whereas but 4.2 per cent were positive by the modified serial section method.

Table 2 below shows a comparison of the digestion and the press methods made incidentally while working with wild rats on another phase of trichinosis. It will be seen that of 8 rat diaphragms positive for trichina by the digestion method in only 3 were trichina detected with the press method—a percentage of 37.5 per cent.

An attempt was made in these cases to correlate quantitatively

^{*} Accurate data on the calcification of encysted trichina, especially in man, are wanting.

the number of trichina found by the digestion and the press methods. In these three cases 13, 25, and 71 per cent respectively of the trichinae found by the digestion method could be detected by careful application of the press method.

TABLE 1

DETECTION OF TRICHINA IN KNOWN INFESTED MATERIAL (HUMAN DIAPHRAGMS).

A COMPARISON OF THE DIGESTION METHOD, PRESS METHOD, AND
MODIFIED SERIAL SECTION METHOD

TOTAL SPECIMENS EX- AMINED (ALL POSITIVE BY DIGESTION METHOD)	POSITIVE BY PRESS METHOD	POSITIVE BY MODIFIED SECTION METHOD	COMPARATIVE PERCENT AGE POSITIVE
			per cent
29	11		38
73		17	23

A quantitative correlation of these data would be useful but cannot now be made.

TABLE 2

DETECTION OF TRICHINA IN KNOWN INFESTED MATERIAL (RAT DIAPHRAGMS).

A COMPARISON OF THE DIGESTION METHOD AND THE PRESS METHOD

TOTAL SPECIMENS EXAMINED (ALL POSITIVE BY DIGESTION METHOD)	POSITIVE BY PRESS METHOD	COMPARATIVE PERCENTAGE POSITIVE PRESS/DIGESTION
		per cent
8	3	37.5

DISCUSSION

In our hands trichina are found about 4 times more often by the digestion method than by a modified serial section method, and about 3 times more often than by the press method. Others have found the section method unsatisfactory³ though Hall¹⁵ urges that the press examination of 1 gram of diaphragm taken from near the tendinous portion be made a routine part of every necropsy. Several factors are concerned in causing this difference of opinion, the chief being, we suppose, the difference in the quantity of infestation of the material studied by Hall and by us. In heavily infested specimens any method of examination reveals the trichina, while in lightly infested specimens the

digestion method reveals the infestation more often than do the other methods.

The results of the digestion of a biopsy must be interpreted with understanding, just as must other laboratory procedures. The presence of cysts would of course make past infestation certain, but in routine postmortem studies from cases in which there was no thought of trichinosis clinically, nor any history of the disease in the past, living larvae are reported in from 56 per cent⁵ to all³ of the positive cases. The preparation of a microscopic section from a portion of the biopsy is of value in the interpretation of the digestion results, since one would expect no myositis in an old infection. Unless these facts are considered, the results of the digestion of a biopsy may be misinterpreted, as the biopsy may contain a few trichinae acquired in the past, though their presence may not mean that the patient is now suffering from trichinosis.

We do not know what the possibility is of a patient having trichinosis with no trichina being present in a given biopsy specimen. This is dependent on two factors, first the number of trichina that are required to produce the clinical disease and second, the localization in the various muscles. Scheifley, in experiments with dogs, has demonstrated that the trichina are rather uniformly distributed through the various muscles which are accessible to biopsy, that is, the muscles of the extremities¹⁶. Unpublished data in this laboratory indicated that the distribution is also fairly uniform in muscles accessible to biopsy in the human as well as in some others (the diaphragm, psoas, rectus abdominis, and muscles of deglutition)¹⁷. We know little concerning the number of trichina per gram of muscle that are needful to produce clinical trichinosis. Hall, on the basis of his 41 positive cases considered that the infestation "resulted in zoological nonclinical trichinosis in about 25 per cent of our cases (those with less than 10 larvae per 100 grams), in what must have been severe clinical trichinosis in at least 4.9 per cent of our cases (those with over 100 larvae per gram) and in clinical trichinosis, more or less atypical, in about 70 per cent of our cases".

Though we have seen several examples of an infestation in man of from 10 to 15 larvae per gram of diaphragm, we could find nothing in the histories available suggestive of a past attack of trichinosis.*

At present two facts are known—in the muscles of patients dying of trichinosis, trichina are usually numerous and easy to find, while the results of examinations of cadavers and bodies in routine necropsies indicate that whereas the incidence of infestation is high, it usually is light in each individual case. So far as we know there have been no quantitative comparisons of the number of trichinae found either at necropsy or by biopsy with the clinical severity of known cases of trichinosis. Such quantitative determinations are urgently needed both to determine the number of trichina necessary to produce clinical manifestations, and if possible to correlate the quantity of the infestation with the clinical severity of the disease. It may be that either the usual section method or the press method is sufficiently accurate to find trichina in biopsies of patients with clinical infestation; however, until quantitative studies are made we cannot be certain of this.

It may not be inappropriate to mention at this point that we are strongly of the belief, on the basis of previous observations, that the control of trichinosis is a public health problem, and that the disease can easily be controlled by the prevention of the feeding of unsterilized garbage to hogs. The slogan of the Bureau of Animal Industry "Cook pork well," while useful, by implication places the responsibility for the prevention of trichinosis upon the housewife. We believe that the onus of trichinosis should be lifted from the housewife, and placed squarely where it belongs—upon the United States Public Health Service.

^{*}In the rat in nature we have seen infestations of as high as 200 larvae per gram, and in the hogs as high as 35 per gram. As many as 3320 per gram have been reported in swine. Chandler reports 2,000,000 larvae per pound (a little over 900 per gram) in sausage responsible for an epidemic of trichinosis in Portland, Oregon. Doses of 5 larvae per gram of body weight are inevitably fatal in the monkey, according to McCoy. Doses of 5 larvae per gram of body weight are inevitably fatal in the monkey, according to McCoy.

CONCLUSIONS

- 1. The laboratory diagnosis of trichinosis is briefly discussed.
- 2. An additional diagnostic procedure for the detection of trichina, namely the digestion in artificial gastric juice of muscle biopsies, necropsy tissue, and meat products is described.
- 3. The digestion method in our experience is four times more accurate for the detection of trichina in known infested specimens than is a modified serial section method, and approximately three times more accurate than the press method.
- 4. The digestion method, complemented with a control microscopic section, is recommended as the diagnostic procedure of choice for the detection of trichina in biopsy and necropsy material, and in suspected meat products.
 - 5. The morbidity of trichinosis is unknown.
- 6. The need for quantitative studies of the number of trichinae in biopsy and necropsy material from known cases of trichinosis is discussed.

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SPIROCHETAL JAUNDICE IN BUFFALO, NEW YORK*

REPORT OF A FATAL CASE IN A FISH MERCHANT

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The following case is presented to establish the existence of Weil's disease in the Great Lakes' port of Buffalo, and constitutes the eighth fatality from this disease reported in the United States accompanied by conclusive clinical pathological evidence. A survey of the literature from Wadsworth's first case report in 1922 to the present time reveals that the most informative review of the literature is now contained in the paper published by Jeghers, Houghton, and Foley in 1935, who reported the fifth fatal case.¹ Additional fatalities have been reported by Kilgore² and Mulholland.³ It appears that the disease is becoming more frequently detected in its milder forms, since a considerable number of such diagnoses have been established by dark field examinations of blood plasma or urine, by guinea-pig inoculation, and by agglutination tests within the past three years⁴.⁵.⁶.².

CASE REPORT

The patient, a 45 year old Greek fish merchant, operating his own business, and having resided in the United States for 25 years, was admitted September 22, 1938 to the Millard Fillmore Hospital acutely ill and with anuria of 48 hours duration. The oxalated blood specimen sent to the laboratory yielded a urea-N of 64 milligrams per 100 cubic centimeters and a creatinin of 6.6 milligrams per cent. Because of the markedly yellow color of the plasma, bilirubin determinations were also made, with the following results: Icterus Index 75; Van den Bergh reaction positive; quantitative bilirubin 7.7 milligrams per 100 cubic centimeters.

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Clinical jaundice had not been observed up to this time. Azotemia and jaundice at this level thus indicated a hepato-renal syndrome, commonly occurring spontaneously almost exclusively in Weil's disease.

Questioning disclosed that he had been well until 5 days prior to admission, when the abrupt onset of the present illness was characterized by a succession of chills for the first two days with a fever of 101°, associated with and followed by intermittent nausea and vomiting, anorexia, malaise, and marked abdominal distention and tenderness. Light colored stools and dark colored urine were described. Occasional blood-tinged sputum had been expectorated. When asked about the proximity of rats, he stated that he had often noted that they had gnawed the packing cases at his place of business. He could not recall any skin injury or splinters and could offer no suggestion as to the mode of infection from drinking water or other sources. Physical examination revealed marked abdominal distention with generalized tenderness and splinting, especially in the right upper quadrant. Cutaneous jaundice, anxiety, dry tongue, and distress were obvious. The temperature was 98.4, the pulse 130, and respirations very slightly accelerated. An area of cutaneous ecchymosis, 3 x 6 centimeters, was found on the upper abdomen just below the ensiform, which had not previously been observed. Several discs on his back from cupping two days previous to admission seemed a bit redder and more hemorrhagic than might be expected.

One internist consultant scouted the inference of Weil's disease, on general principles; another considered an operative emergency to exist and advocated immediate laparotomy. The family physician decided against operation.

Since the jaundice had already developed to a high level, the urinary bladder was catheterized and a half-ounce of urine obtained for guinea-pig inoculation in preference to blood.

The patient died on the tenth day of illness, (fifth day of hospitalization).

On his first day of hospitalization he was unable to retain anything in his stomach. Palliative measures afforded some relief. His blood pressure was 110/60. Additional laboratory findings were as follows: Hemoglobin 75 per cent (Sahli); red count 3.5 million; white count 11,000; polymorphonuclears 90 per cent; blood sugar 125 milligrams per 100 cubic centimeters. Kline exclusion, Kline diagnostic, and Kahn standard diagnostic tests were all negative. Urine showed a trace of albumin, no casts, 5 leukocytes, and 15 red cells per high power field.

On the second day the temperature was 99, the pulse 128, and respirations 38. Nausea and distention continued. A stomach wash obtained considerable dark brown fluid with "coffee grounds," and a duodenal intubation obtained grossly bloody fluid. Serum bilirubin tests were as follows: Icterus Index 100; Van den Bergh reaction positive; quantitative bilirubin 14 milligrams per 100 cubic centimeters.

On the third day the temperature rose to 103, the pulse dropped from 140 to 120, and respirations were 36. He could take a little water by mouth, although

still nauseated and distended. Frequent bowel movements were passed, black in color, and containing blood clots. He became confused, irrational, and restless. Blood pressure 140/50; urine output nil.; blood chlorides 458 milligrams per 100 cubic centimeters; hemoglobin 62 per cent; red count 3.3 million; white count 12,000; polymorphonuclears 67 per cent.

On the fourth day his temperature was 99, pulse 118, and respirations 30. Seemed a little more rational during the night. Urine output 1½ ounces. Expectorating a considerable amount of bloody mucus. Right arm swollen. Jaundice deepening visibly. Hemoglobin 53 per cent; red count 2.5 million;

white count 14,200; polymorphonuclears 88 per cent.

On his fifth day of hospitalization, his temperature was 100; his pulse had dropped progressively from 120 at noon of the fourth day to 30 at death; respirations were 40. Delirium and coma preceded death. An indirect blood transfusion was given an hour and a half before death. Icterus index 250; Van den Bergh reaction positive; quantitative bilirubin 35 milligrams per 100 cubic centimeters. Sedimentation rate 30 millimeters in 10 minutes, 36 millimeters in 20 minutes (no further drop). Urine showed a cloud of albumin, no sugar or acetone, many hyaline and granular casts, and numerous white and red blood cells. Blood Group A. Permission for autopsy was not granted.

Results of guinea-pig inoculations

Pig no. 1: This white pig was inoculated subcutaneously in the groin, rather than intraperitoneally, with urine obtained by catheter on the day of admission. Until the 12th day the animal exhibited little, if any, indisposition, but on the 12th day jaundice became readily demonstrable in the ears, sclerae, mucous membranes, feet, and skin. The pig died suddenly on the 13th day with intense generalized icterus, which was quite spectacular. At autopsy several areas of hemorrhage were noted in the lungs, and the under surface of the skin appeared very yellow, but the other organs, including the adrenals and kidneys, did not appear grossly remarkable. Hematoxylin and eosin preparations showed a few foci of somewhat caseous necrosis scattered throughout the liver without a predilection for any particular lobular zone. Other findings were not especially significant in addition to the gross. A modified Levaditi stain showed the liver literally swarming with typical leptospirae in "C" and "S" forms with the characteristic tapering hooked ends, and a fairly generous sprinkling of them in the renal tubular epithelium. A microphotograph of any field in these liver sections would be equally as representative as the one presented with this report.

Dark field examination of the patient's urine was not convincing prior to guinea-pig inoculation nor could the spirochetes be demonstrated in the pig's blood or urine by this method.

Pig no. 2: This white pig was inoculated subcutaneously in the groin with the urine aspirated from the bladder of the first pig at the autopsy, and it died in the same manner on the 10th day with similar gross and microscopic findings,

but with an empty urinary bladder. A blood count just before death gave the following findings: Red count 5.2 million; White count 8200; Neutrophils 70 per cent; Basophils 4 per cent; Lymphocytes 24 per cent; Monocytes 2 per cent. Serum bilirubin determinations on blood expressed from the heart and great vessels immediately after death were as follows: Icterus Index 250; Vanden Bergh reaction positive; Quantitative bilirubin 25 milligrams per 100 cubic centimeters. Dark field examination of the serum of this pig failed to disclose any typical organisms. Plasma was not examined.

Pigs nos. 3, 4, 5: These animals were inoculated with the urine of the patient, after it had stood at room temperature 23 days, heart's blood of pig no. 2, and a bladder emulsion from pig no. 2, respectively. No reaction of any significant nature has been noted in 12 weeks of observation of these three animals other than a neutropenia in the pig inoculated with the old urine specimen. On the fourth day the blood count of this pig showed: Hemoglobin 103 per cent; red count 5.5 million; white count 4750; neutrophils 12 per cent; lymphocytes 87 per cent; monocytes 1 per cent. On the seventh day the following blood count was obtained: Hemoglobin 113 per cent; red count 6.4 million; white count 9,000; neutrophils 25 per cent; lymphocytes 75 per cent.

The association of the wild rat with the transmission of Weil's disease to man has become well established, although the exact mode of infection is often undeterminable. It is estimated that 10 per cent of these animals, although not subject to the disease themselves, harbor the organisms and excrete them in abundance in their urine and saliva, thus contaminating water, especially if it is somewhat stagnant. Hence the disease is commonly reported in various parts of the world among coal miners, sewer workers, fish handlers, bathers, slaughter house workers, grooms, rice field workers, soldiers occupying undrained trenches, bargemen, butchers, and garbage handlers. The case presented herein is the third reported in a fish handler in the United States⁵.

Although clinical jaundice is said to develop in only 60 per cent of cases in general, it has been demonstrable in all fatal cases reported so far in the United States. In many recovered cases it could undoubtedly be demonstrated by the serum bilirubin tests when not definitely manifested in the skin or sclerae. It is doubtful if the causative organism can be present in the blood stream without also being present in the urine, although after the jaundice has developed it usually disappears from the blood

and can be found only in the urine. This phenomenon might be associated with the observation of Noguchi that the leptospira icterohemorrhagica is bile-soluble. Possibly some interfering substance inhibits this mechanism in the tissues. For confirmation of the diagnosis when dark field examinations of blood plasma or urine, or guinea-pig inoculations fail to show the presence of the organism, agglutination tests may be performed after the first week or ten days, in which titres of 1:30,000 have often been reported.

The jaundice of this particular case is of some interest. Since the first icterus index was 75 and the bilirubin level 7.7 at the same time, ascending jaundice was implied because the bilirubin value exceeded a tenth of the icterus index value, and subsequent findings proved this point. Furthermore, the terminal bilirubin level of 35 milligrams per 100 cubic centimeters of serum is the highest yet encountered in any icterus observed by the writer.

In the tissues of the guinea-pigs the microscopic findings, aside from the generalized presence of the leptospirae, were remarkable only for scattered foci of caseous liver necrosis in which typical organisms were not found, although fragments appeared as if lysis had occurred. The complete absence of bile retention in the polygonal cells of the liver was rather startling. The rapidity with which the jaundice developed in the pigs, and the consistent disturbance of the bilirubin-icterus index ratio (more than 1-10) during the ascent of the jaundice in the patient imply that the jaundice may well be termed "dynamic" in response to a potent icterogenic factor bringing about an almost complete terminal suspension in function of the bilirubin excretion mechanism in the liver. Theoretically this would imply the development of a state of complete impermeability of the Kupffer cells to circulating pigment. Further studies on jaundice in guinea-pigs should prove interesting.

The study of the position of the spirochetes in the tissues examined revealed a general distribution in lymphatic channels, parenchymatous cells, and lumens of the renal tubules especially in the Henlé loops. In the liver, although commonly intracellular, many fields showed a tendency to localization along the borders of polygonal cells, accentuating the cell outlines, and also in perinuclear positions accentuating nuclear outlines. In the kidneys they were frequently seen investing the luminal surfaces of the epithelial cells of the Henlé loop region, forming a complete ring around the lumens, evidently in process of entering the tubules. An occasional organism could be seen in the glomeruli. In the lung, they were present in small numbers among the red cells in alveoli, and in hemorrhagic areas.



Fig. 1. Leptospira icterohemorrhagica in liver of guinea-pig

SUMMARY

The case presented constitutes the eighth proven fatality from Weil's disease reported to date in the United States, the third case of the disease known to have occurred in a fish handler in this country, and the first proven occurrence in the Great Lakes port of Buffalo, New York. The disease which ran its course in ten days was recognized clinically by its sudden onset with chills, anuria, generalized muscle pains (especially of the abdominal wall), jaundice fever, prostration, vomiting, cough, and hemorrhagic manifestations. From the clinical pathologic

point of view the hepato-renal syndrome was established by marked azotemia and ascending juandice of great intensity, by the typical death of a guinea-pig 13 days after subcutaneous inoculation in the groin with urine obtained from the patient on the sixth day of illness, and the typical death of a second guinea-pig ten days after inoculation with the urine of the first pig, obtained at autopsy on that animal. Levaditi stains demonstrated classical spirochetes in enormous numbers in the livers of both animals, as well as their abundance in the kidneys.

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THE SERUM PHOSPHATASE IN THE DIFFERENTIAL DIAGNOSIS OF OBSTRUCTIVE JAUNDICE*

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For years the clinical pathologists have searched for a practical laboratory procedure by which they could differentiate obstructive from non-obstructive jaundice. As a result, various methods have been announced from time to time. In this country, the Van den Bergh bilirubin test and the galactose liver function test have enjoyed the greatest popularity. Time has proved both to be unsatisfactory for accurate differentiation of jaundice.

Recently Roberts⁵ noted that in obstructive jaundice the serum phosphatase values were above ten units, while in non-obstructive type the values were below ten units. Subsequently Rothman, Meranze, and Meranze⁶ studied a large series of clinical cases of jaundice and confirmed Robert's original observations. Rothman and his associates classified their cases of jaundice as follows:

"1. Obstructive jaundice, i.e., jaundice due to extra hepatic obstruction due to stones in the common bile duct or due to an obstructing neoplasm involving the common bile duct, etc.

"2. Hepato-cellular or non-obstructive jaundice, i.e., jaundice resulting from direct injury to the liver cells or to cholangitis such as in the so-called acute catarrhal jaundice.

"3. Hemolytic jaundice."

The normal range suggested by these authors is six to ten units for adults and six to sixteen units for children.† In addition, they observed a definite relationship existing between serum phosphatase and serum bilirubin in obstructive jaundice, the

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† In a recent communication with D. Meranze,⁴ the author states that adult figures below twelve definitely favor a diagnosis of hepato-cellular jaundice and above sixteen units definitely favor a diagnosis of obstructive jaundice.

rise in phosphatase parallelled the rise in serum bilirubin until a limit of phosphatase value was reached. In non-obstructive jaundice the phosphatase levels did not exceed ten units despite progressive increase in bilirubin.

The present observations were made from a series of forty-one cases of jaundice. The method of phosphatase determination and the system of classification for the cases of jaundice are the same as suggested by Rothman and his co-workers, with the exception that there has been added to the obstructive group a number of cases which, from the anatomic and physiologic point of view, should be regarded as obstructive types.^{1,2} This group includes all the cases of cholecystitis with spasm of the sphincter of Oddi producing a dilatation of the common duct and jaundice. The serum bilirubin test determinations were made by a photometric method modified in our laboratory.³

RESULTS

Forty-one cases of jaundice were analyzed and the diagnoses proved either by operation or autopsy in fifty-three per cent of

TABLE 1

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	TOTAL NUMBER OF CASES	NUMBER OF CASES OF AGREE- MENT	PERCENT- AGE AGREE- MENT	NUMBER OF CASES OF DISAGREE- MENT	PERCENT- AGE DIS- AGREE- MENT
			per cent		per cent
Hepatocellular jaundice:					
Catarrhal jaundice	7			7	
Toxic hepatitis		6		1	
Cirrhosis of liver	6	6			
Total	20	12	60.0	8	40.0
Obstructive jaundice:					
Cancer of head of pancreas		3			
Choledocholithiasis	2	1		1	
Cancer of bile ducts	2	2			
Obstruction of common					
duct	4	2		2	
Cholecystitis with sphincter					
spasm	10	7		3	
Total	21	15	71.4	6	28.6
Total	41	27	65.9	14	34.1

the cases. The diagnoses in the remainder of the cases were made on a clinical basis. If the cases were obstructive in type and the phosphatase determinations were above ten units, these were classified as agreement cases; if below ten units, as disagreement cases. If the cases were hepato-cellular in type and the phosphatase determinations were above ten units, they were classified as disagreement cases; if below ten units, as agreement cases. Of the forty-one cases analyzed (table 1) twenty-seven cases or sixty-five and nine-tenths per cent were agreement cases and fourteen or thirty-four and one-tenth per cent were disagreement cases.

DISCUSSION

It is of interest to note that all the phosphatase determinations in the so-called catarrhal jaundice group were above ten units and must be considered as disagreement cases according to the criterion cited above. If these cases as a whole were transferred to the obstructive division, they would then be agreement cases. In reviewing the pathologic features of catarrhal jaundice, one finds that it cannot be considered a pathological entity and therefore, cannot be definitely placed either in the hepato-cellular group or in the obstructive group. The older workers believed that catarrhal jaundice was due to an ascending infection of the bile ducts arising in the duodenum, or that it was due to an inflammatory occlusion of the finer biliary ducts in the liver. Present day opinion holds that the damage is due to the liver itself, or a variable degree of acute necrosis is the essential lesion of the disease, "a minature acute yellow atrophy." The question is then raised, are we sufficiently informed to classify catarrhal jaundice in one or the other groups?

Of the two remaining groups in the hepato-cellular division, it is noted that all cases of cirrhosis of the liver are in agreement. Likewise, all cases of toxic hepatitis are in agreement with the exception of one (No. 11, table 2), a case of sulphanilamide intoxication with granulocytopenia, which, at autopsy, was found to have severe parenchymal liver damage. The phosphatase

reading in this case was 23.8 units and must, therefore, be considered a case definitely in disagreement.

Of the twenty-one obstructive cases, fifteen, or seventy-one and four-tenths per cent, were in agreement and six, or twenty-eight and five-tenths, were in disagreement. In this group are included ten cases of cholecystitis with or without cholelithiasis with obstruction of the sphincter of Oddi. Seven of these cases were in agreement and three were in disagreement. The majority of these cases showed acholic stools, definite evidence of a complete obstructive phenomenon. With subsidence of inflammation and release of spasm of the sphincter, the jaundice cleared in all these cases.

Of the remaining cases including carcinoma of the head of the pancreas, carcinoma of the liver and bile ducts, stones in the common ducts, all were in agreement with the exception of two cases. In one (no 5, table 3), autopsy revealed congenital absence of the gall bladder and a stone in the common bile duct. Previous to death, the jaundice increased progressively with the serum bilirubin reading 13.3 mgm. and the phosphatase reading In the other case (no. 14, table 3), the autopsy revealed congenital atresia of the common bile duct in a new-born which showed progressive jaundice until death at the age of ten months. The serum bilirubin was 35.5 mgm. per cent and the phosphatase 9.2 units. As an explanation for these cases, we cite the very interesting and valuable contribution made by Thannhauser, Reichel, Grattan, and Maddock. Experimentally, they have demonstrated that the increase in blood phosphatase in obstructive jaundice is due, not to an increase in the amount of enzyme, but due to an increase of activity of the enzyme by substance in the bile similar to ascorbic acid, and that bile acids, on the contrary, depress the activity of phosphatase. Moreover, their experiments on dogs with bile fistulae revealed the unexpected results of phosphatase values after operation ten to twenty times as high as the initial values. Ascorbic acid was found to activate blood phosphatase of bile fistula dogs to the same extent as dogs with common duct ligation. These

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TABLE 2
HEPATOCELLULAR JAUNDICE

HERMUN	CLINICAL DIAGNOSIS	CONFIRMED	BILL- RUBIN	CNITS OF PHOS- PHA- TASE	AGREEMENT OR DISA- GREEMENT	REACTION	OBSERVATIONS
1			mgm. per cent				
-	4 Catarrhal jaundice		5.4	30.3	D*		Chills, fever, malaise. Diabetic. Bile in urine. No
10	15 Catarrhal jaundice		6.0	6.0 15.4	Q	Positive F.	clay colored stools Voniting, headache, jaundice, slight abdominal pain.
	16 Catarrhal jaundice		7.9	13.8	D	Positive	Onset with cough, fever, pain in right side. Bile in
~	28 Catarrhal jaundice		6.3	28.5	D	Positive Di	urine. Acholic stools Chills, fever, pain in back and chest, voniting, loss of
-	30 Catarrhal jaundice		2.2	18.0	D	Positive For	Age 9 years, pain in abdomen, vomiting. Jaundice
00	38 Catarrhal jaundice		13.3	36.6	D	Positive Direct	gradually cleared. Hemolytic Strep. in throat Chills, fever, malaise. Jaundice cleared
	36 Catarrhal jaundice		2.5	14.5	D	Positive	Vomiting, progressive jaundice, which cleared after
	11 Toxic hepatitis, sulphanilamide intoxication	Autopsy 7.4	7.4	23.3	А	Positive Direct	Wbc. 1,500, with complete disappearance of polys. Urine: bile negative. Autopsy: diffuse hepatitis
1	H		8.45	3.6	Aţ	Positive Direct	Hemolytic streptococcic pharyngitis. Hemoglobinuria. Urine: bile negative
00	Toxic hepatitis		26.5	7.0	A	Positive	Lobar pneumonia confirmed by X-ray. Bile in urine
6	Toxic hepatitis, cere- Autopsy 1.8 bral hemorrhage	Autopsy	1.8	7.9	A	Positive Direct	Autopsy: mitral endocarditis. Cerebral hemorrhage

10	Toxic hepatitis, rup-	Autopsy	0.6	4.3	Y	Positive	10 Toxic hepatitis, rup- Autopsy 9.0 4.3 A Positive Autopsy: generalized peritonitis. Marked degenera-
	tured duodenal ulcer		4.8	5.8		Direct	tive change in liver and kidney
19	19 Cirrhosis of liver	Autopsy	0.7	5.8 A	¥	Negative	Negative Cholecystectomy 1933. Autopsy: roughly granular
R	23 Cirrhosis of liver	Autopsy 2.0 5.8 A	2.0	5.8	A		Chronic alcoholic. Autopsy: marked cirrhosis of liver.
31	31 Toxic hepatitis, B.		1.6	1.6 9.9 A	V	Positive	Positive Osteomyelitis of right hip with gas bacillus infection
33	Welchii infection 33 Septic abortion, toxic	Autopsy 36.9 5.7 A	36.9	5.7	A	Direct Positive	Autopsy: hepatitis
34	hepatitis 34 Cirrhosis of liver		14.0	14.0 10.3 A	V	Direct Positive	Alcoholic addict. Bile in urine
37	37 Cirrhosis of liver lues	Autopsy 20.6 7.5 A	20.6	7.5	A	Direct Positive	Direct Positive Autopsy: portal cirrhosis with rupture of esophageal
			17.0	6.7		Direct	varices and fatal hemorrhage. Gumma of liver. Wassermann 4+
39	39 Traumatic injury		11.5	11.5 6.0 A	¥	Positive Direct	Hemorrhage following injury sustained in automobile accident
9	40 Cirrhosis of liver		11.3	11.3 7.5 A	4	Positive Delayed	Recurring painless jaundice. No fever. Erythrocytes uniformly large

* Disagreement.

TABLE 3
OBSTRUCTIVE JAUNDICE

CVEE	CLINICAL DIAGNOSIS	CONFIRMED	BILL- BUBIN	UNITS OF PHOS- PHA- TASE	OREEMENT OR DISA-	BEACTION	OBSERVATIONS
			mgm. per cent				
co	Cancer of common bile	Autopsy	10.0	24.7	A *	Positive	Clay colored stools; loss 75 lbs. of weight. Autopsy:
	duet					Direct	cancer of common duct with metastasis to regional
9	Cancer of gall bladder	Oneration	23.5	22.5	V	Positive	Progressive painless jaundice
)		- I				Direct	
10	Choledocholithiasis	Autopsy	13.3	6.7	Dţ	Positive	Intermittent attacks of jaundice. Bile in urine.
						Direct	Autopsy: congenital absence of gall bladder.
							Cirrhosis of liver. Choledocholithiasis
14	14 Congenital atresia of Autopsy	Autopsy	35.5	9.5	Q	Positive	Ten month child with increasing and progressive
	bile ducts					Direct	jaundice, ascites, edema, and loss of weight. Au-
							topsy: rudimentary common bile duct. Cirrhosis
							of liver
13	13 Obstruction of bile	bile Operation 21.7	21.7	8.2	Q	Positive	Progressive jaundice; loss 30 lbs. of weight; clay
	duct					Direct	colored stools. Operation: liver smooth, stone
							palpated
18	Obstruction of com-		15.7	25.0	A	Positive	Progressive jaundice, 8 weeks duration, acholic
	mon duet					Direct	stools, loss of weight. No pain
22	Stricture of	common Operation	0.6	23.8	A	Positive	Cholecystectomy one year ago. Progressive jaundice
	bile duct					Direct	for past three months. Operation: stricture of
							common duct
1	1 Choledocholithiasis	Operation	13.2	23.3	A	Positive	Operation: stone in common bile duct with perfora-
			16.2	29.7		Direct	tion
			14.0	9.7			
			6.7	12.2			
29	29 Cancer of head of pan- Operation creas	Operation	14.3	29.0	V	Positive Direct	Operation: common duct dilated. Pancreas, hard, firm, nodular. No stone palpated
					-		

26 Cancer of head of pan- Operation 8.4 36.5 A Positive Liver palpable 4 fingers below costal arch. Operation:

	SER		PH	e e	PHA	TASI	E IN	OF	STI	LU (CTI	VI	E J	AU]		ICE	m	y:		233
Liver palpable 4 fingers below costal arch. Operation: common bile duct 2 cm. diameter obstructed by mass in head of pancreas. Biopsy	Operation: gall bladder enlarged. No stones. Firm nodular mass in head of pancreas	Severe pain in right upper quadrant radiating to back,	nausea, Vomiting, tenderness right costal margin. Bile in urine	Pain in right upper quadrant, gastric distress. Bile	in urine. Jaundice disappeared Jaundice, diarrhea, selective dyspepsia, fatigue.	Gall bladder drained 1 year ago. Clay colored stools. X-ray: non-functioning gall bladder	Operation: liver enlarged. Gall bladder engorged; stones present		Gall stones palpated, but not removed. Liver small	_	mon bile duct opened, but no stones found. Clay	colored stools	Operation: cholelithiasis		Sudden onset of crampy right hypochondriac pain	radiating to back, nausea, vomiting, clay colored stools	Operation: gall bladder bound down to bed with firm	mass replacing proximal \ of gall bladder. Biopsy: inflammatory tissue	X-ray: calculus within gall bladder shadow	
Positive Direct	Positive Direct	Positive	Direct	Positive	Direct	Direct	Positive Direct	Positive	Direct	Positive	Direct	:	Positive		Positive	Direct	Positive	Direct	Positive	Direct
<	A	A		A	A		A	V		A			9		A		Q		A	
36.5	16.1	20.0		16.2	25.0		26.6	15.4		7.0 18.2	25.2		7.0		8.1		7.0		4.5 12.9	
œ 4.	10.5	4.2		4.7	2.6			5.5		7.0	7.95	•	1.0		21.5		6.2		4.5	
Operation	Operation						Operation	Operation		Operation			Operation				Operation			
of pan-	of pan-	with	sm	with	sm	sm		with	sm	with	assoc.	r spasm	with	r spasm	with	sm	with	sm	with	
Cancer of head of pan-	Cancer of head of pan-Operation creas	Cholecystitis	sphincter spasm	Cholecystitis	sphincter spasm Cholecystitis	sphincter spasm	Cholelithiasis	5	sphincter spasm	O	cholelithiasis	with sphincter spasm	Cholecystitis	with sphincter spasm	Cholecystitis	sphincter spasm	Cholecystitis	sphincter spasm	Cholecystitis	cholelithiasis
26	25	12		20	63		24	32		41		1	17.		17		21		35	

cancer of near of pair Operation 17.0 20.0 A Direct firm, nodular. No stone palpated

* Agreement.

observations tend to show the fallacy of attempting to differentiate obstructive from non-obstructive jaundice by an arbitrary phosphatase level, since the accumulation of bile salts can decrease activity and give erroneous results. In addition, we have yet to explain the high phosphatase value in bile fistula dogs.

SUMMARY AND CONCLUSIONS

1. A series of forty-one cases of jaundice has been investigated and classified according to the method of Rothman, etc., to determine the value of serum phosphatase in differentiating obstructive from non-obstructive jaundice.

2. Sixty-four per cent of the cases were in agreement and thirty-six per cent were in disagreement.

3. A new classification is suggested to be included in the obstructive jaundice group; namely, that of cholecystitis with or without cholelithiasis accompanied by spasm of the sphincter of Oddi. Seventy per cent of these cases were in agreement and thirty per cent were in disagreement.

4. The question is raised whether catarrhal jaundice can be classified as strictly a hepato-cellular or as an obstructive type of jaundice.

5. The groups including the cirrhoses, carcinoma of the head of the pancreas, and carcinoma of the bile ducts, are in agreement.

6. We conclude, therefore, at the present time, the serum phosphatase determination cannot be reliably used as a procedure for differentiating obstructive from non-obstructive jaundice per se, but it may be so in the future, when all the factors governing the activity of the enzyme phosphatase are known and when all types of jaundice can be classified on a pathologic basis.

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TORULA MENINGITIS IN A CHILD*

RIGNEY D'AUNOY AND CHARLES R. LAFFERTY

From the Department of Pathology and Bacteriology, State Charity Hospital of Louisiana at New Orleans, and the School of Medicine of Louisiana State University

Meningitis, produced by the yeast-like organism, Torula, is comparatively rare. With a single exception all of the sixty-three cases thus far reported^{1,2,3,4,5} occurred in persons over ten years of age; the majority being between thirty and sixty. The only case afflicting a child, a girl three years and four months of age, was observed by Barlow in Australia (1923)⁶. The case here presented occurred in a negro child and is incidentally the second case of Torula meningitis ever reported from the State of Louisiana⁷.

REPORT OF A CASE

The patient, a negro boy, seven years of age, a native of Louisiana, was admitted to the State Charity Hospital of Louisiana at New Orleans, on February 12, 1937. No history was obtainable. On admission he appeared well developed, fairly well nourished, acutely ill, irritable and in a semi-comatose state. His temperature was 102°F., his pulse was 120 and his respirations 24 per minute. The neck was rigid. The chest and abdomen revealed nothing remarkable. The reflexes of the lower extremities were lively. Kernig's sign was present. The impression was that the child had meningitis. The cerebrospinal fluid was under increased pressure; each of four specimens taken at six hour intervals was clear, had globulin from a trace to one plus and 100 to 250 cells with lymphocytes predominating. No organisms were detected in the smears. Dextrose brain broth and dextrose brain agar inoculated with the cerebrospinal fluid on the 12th and the 13th of February yielded yeast-like cells compatible with Torula. The patient's condition grew progressively worse and he expired forty-eight hours following admission.

At necropsy (limited to examination of the cranium) the dura mater was tense, the leptomeninx cloudy and somewhat thickened. The cerebral and cerebellar hemispheres, the pons and the medulla oblongata were proportionate

^{*}Received for publication May 12, 1938.

and symmetrical. The convolutions of the cerebral hemispheres were flattened and the sulci narrowed. There was a slight pressure cone over the cerebellum. The blood vessels were distended and along their courses there were scattered yellow white tubercle-like nodules measuring up to 2 mm. in diameter. These were more numerous at the base where a fibrinopurulent exudate covered the pons and the medulla oblongata. The cut surfaces of the cerebral hemispheres disclosed no changes in the gray or white matter. The cavities of the right and left ventricles and the third ventricle were not noticeably increased in size, nor were there any changes in the choroid plexuses. The surface of the enpendyma seemed slightly granular. No changes were noted on the cut surfaces

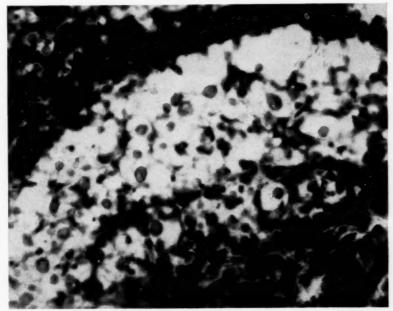


Fig. 1. Yeast-like bodies (Torula histolytica) in the exudate of the pia arachnoid of a seven year-old negro boy, who died of torula meningitis.

of the cerebellar hemispheres, the pons and the medulla oblongata. The aqueduct of Sylvius and the cavity of the fourth ventricle were not increased in size.

Routine microscopic preparations from various parts of the cerebral cortex, the cerebellum and the pons disclosed no changes in the brain substance proper, although in the wall of some of the small blood vessels there were marked subendothelial infiltrations of small round cells and large mononuclear cells. The tissue elements of the pia arachnoid were spread apart and the spaces filled with fibrin, a cellular debris, some small round cells, polymorphonuclear leukocytesmany large mononuclear cells and occasional giant cells. Scattered through,

out, but more numerous in some areas than in others, were clear round or oval bodies of the size of a large mononuclear cell, contoured by a sharp, narrow, dark line. Each body was surrounded by a light halo.

Smears from the exudate covering the pons disclosed yeast-like bodies identical in appearance with those cultured from the cerebrospinal fluid. Dextrose brain broth, brain agar and gelatin slants, potato and carrot media inoculated with the exudate yielded an abundant growth of the same organism. No mycelia or spores were observed.

Four adult white mice inoculated intraperitoneally with 0.5 cc. each of a suspension containing the organisms remained well until the seventh day, when they became weak and their fur roughened and thinned. They died two days later. Smears and cultures from the peritoneal fluids, the livers and lungs disclosed the yeast-like bodies seen in the original cultures. Three adult male rabbits and three adult male rats inoculated intraperitoneally and intracranially remained alive and well for six weeks. At this time cultures from the liver, spleen and lungs revealed no yeast-like organisms.

Thus the morphologic, cultural and biologic reactions of the organism conform to those of Torula histolytica.

SUMMARY

A case of meningitis produced by the yeast-like organism, Torula histolytica, in a negro boy, seven years of age, is presented. This is the second case ever recorded occurring in a child.

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GENERAL HOSPITAL LABORATORY COSTS*

W. P. STOWE

St. Luke's Hospital, San Francisco, California

Wide divergence of opinion as to what constitutes a fair charge for laboratory services in a hospital and the paucity of reported surveys of the actual costs upon which such charges should be based, prompted this study and analysis of costs as they occur in what may be considered an average sized general hospital.

1. Hospital. The hospital surveyed is a compact four story hospital of 200 beds located well out of the high rent district of San Francisco. Admissions approximate 5500 per year and total laboratory examinations are about 30,000 per year. Admittance urinalyses and complete counts are absolutely required. Wassermanns are optional with the physician. The hospital is fully approved for nurses' training, interne and resident training in specialties and for training laboratory technicians.

2. Space. The general laboratory occupies 650 square feet of The basal metabolism and electrocardiograph room floor space. takes up an additional 150 square feet. Surgical specimen storage and autopsy room are in the basement, sharing the mortuary, and are not considered here as productive or rentable space. Heerman¹, in a careful analysis of the relative value of the rentable floor space in hospitals and in equivalent medical office buildings, shows the former to be worth about three and one half times as much as the latter for floor space and about five times as much as office buildings when gas, electricity, heat, water, janitor service, telephone, bookkeeping and collection are furnished. His figure of \$7.65 per square foot per year for serviced floor space was therefore accepted, the extremely compact laboratory quarters in this hospital being considered to compensate for the greater construction costs and land value of his

^{*}Received for publication October 5, 1938.

hospital. Space costs figured out therefore, at \$4,972.00 per year for the general laboratory and \$1147.00 for the metabolism-cardiograph room (laboratory B).

3. Equipment. This is considered as covering all apparatus and glassware not used up in the tests. Separate inventories of two people established a present value of \$3500.00 for the general laboratory and of \$2000.00 for laboratory B. The "annual value," or lease value, of this is figured at 20 per cent for depreciation plus 6 per cent for interest, or \$910.00 per year for laboratory A and \$520.00 for laboratory B.

4. Total lease value. This is the sum of the space value plus the annual equipment value, here \$5882.00 for the general laboratory and \$1667.00 for laboratory B. In the general laboratory this represents two-thirds the amount of the combined time and materials cost and is so pro-rated into the various tests and procedures, after their time and material costs are determined.

Technicians' time only is here considered in the cost If a full time pathologist is hired on a salary basis his time will be divided into about three equal parts. First oversight and direction of the general laboratory; second, tissue and autopsy pathology and third, teaching, research, consultation and the like. If one-third the pathologist's salary be added here to general laboratory costs, the time cost should be increased one third, and the total cost one-fifth, above the figures given in tables 2 and 3, for each test. Technicians' time is here figured by actual timing of each procedure, as carried out on a patient at average distance from the laboratory, and the actual cost of that time computed according to the salary of the technician capable of and accustomed to performing that test. nicians are employed at salaries ranging from \$100.00 per month to \$250.00 per month. A month is considered 200 hours work, so time costs from fifty cents to a dollar and a quarter per hour. Unproductive time, such as centrifugation or filtration, when other work is being done, is not included.

6. Material. This includes chemicals, reagents, culture media, and antigens actually used up in the tests. With the exception of a few antigens and rare culture media all solutions and reagents

are prepared in the laboratory. The cost of the time required in preparation is included in this column as part of the material cost. Totals of these various cost factors are given in table 1 in approximate thousands of dollars per year, for this hospital, to illustrate the ratio of these factors to each other and to the total costs in the main laboratory.

The high cost of time compared to equipment shown in this table emphasizes the economy of furnishing an abundance of the

TABLE 1
DISPERSION OF LABORATORY COSTS

	MATERIAL	TIME	SPACE	EQUIPMENT	TOTAL
Technician time only	1	9	5	1	16
With 1 pathologist's time		12	5	1	19

TABLE 2
Cost in Cents

PROCEDURE	MATERIAL	TIME	SPACE AND EQUIPMENT	TOTAL
Urinalysis—routine	1	9	6	16
Complete blood count	1	45	30	75
Blood sugar		52	33	87
Sputum—TBC	2	38	24	64
Wassermann (in sets of 30 +)	1.5	27.2	17.3	46
Kahn or Hinton (in sets of 30 +)	1.0	13	8	22
Pneumococcus typing (Neufeld)	130	150	170	450
B.M.R. (technical only)	3	112	165	280
E.K.G. (technical only)	20	150	350	520
Autopsy (with pathologist time)	100	2000	545	2645

latter when it will save time to do so. To force technicians to wait for the use of a microscope or centrifuge because the initial cost of an additional one may be two hundred dollars is poor business. Shaking pipettes for 6000 blood counts, three minutes each, will cost 300 technician hours a year (\$250.00). A thirty-five dollar shaking machine will do the same task better for less than ten dollars a year. Location of the laboratory in the basement, or on the roof or in a separate building will also waste one to several thousand dollars a year in time going to and

TABLE 3

PROCEDURE	COST	UNITS († DOLLAR)	5 × cost)
Urinalysis—routine	\$0.16	1	\$0.75
Sugar only—qualitative	0.05	1 3	0.25
Sugar only—quantitative	0.40	$2\frac{1}{2}$	1.25
Phenolsulphonephthalein		21/2	1.25
Mosenthal	0.25	11	1.00
Blood—complete count	0.75	5	3.00
White and differential	0.53	3-	3.00
Red and hemoglobin	0.30	2	1.50
Typing—per person	0.52	3	1.50
Matching	0.48	3	1.50
Fragility	2.90	17	9.00
Platelet (Gram method)	0.75	5	2.50
Reticulocyte	0.97	6	3.00
Sedimentation	0.35	2	1.50
Coagulation time	0.34	2	1.50
	0.34	2	1.50
Bleeding time		1	3.00
Blood chemistry—sugar	.87	51	
Urea	1.62	10	5.00
Non-protein nitrogen	.87	51/2	3.00
Uric acid	2.72	17	8.00
Creatinine	0.80	5	3.00
Cholesterol	3.85	23	10.00
Chlorides	0.95	6	3.50
Calcium	2.92	18	10.00
Phosphorus	2.00	12	7.50
Glucose tolerance	3.20	23	10.00
Blood—CO ₂ combining power	0.99	6	4.00
Icteric index	0.50	3	2.00
Van den Bergh	0.50	3	2.00
Takata—Ara	1.10	4	4.00
Vitamin C	1.00	4	4.00
Serology-Widal-3 antigens	3.25	20	10.00
Wassermann-sets of 30 +, two antigens	0.46	3-	1.50
Kahn or Hinton—sets of 30 +	0.22	11/2	1.00
Wassermann and Kahn-sets of 30 +	0.52	31/2	2.00
Laughlen—single test	0.95	6	3.00
Heterophil agglutination	1.44	9	5.00
Bacteriology—nose or throat culture	0.15	1	.75
Simple one tube culture	0.15	1	.75
Sputum—tubercle bacilli	0.64	4	2.50
Smear—gonococcus etc	0.42	3	2.00
Autogenous vaccine	2.95	18	10.00
Blood cultures		6-36	3.00-25.00
Stool culture		2-48	1.00-25.00
Pneumococcus typing—Neufeld	4.50	27	15.00
Dark field examination		18	10.00
Guinea pig inoculation (including 6 weeks care	3.00	10	10.00
Guinea pig moculation (including 6 weeks care	3.40	20	10.00

TABLE 3-Concluded

PROCEDURE	COST	UNITS († DOLLAR)	CHARGE (3 TO 5 × COST)
Miscellaneous—Friedmann	3.00	18	\$10.00
Exudates and transudates (culture extra)	.69	4	2.50
Spinal fluid:			
Cell and globulin	0.99	6	3.00
Colloidal gold	0.93	51	3.00
Wassermann -3 am't	1.20	7	3.00
Gastric analysis:			
Single	0.53	3	2.00
Fractional—obtaining	3.45	21	10.00
Testing—6 specimens	2.45	15	7.50
Stool:			.,
Ova only	0.24	11	1.00
Microscopic and blood	0.41	21	1.50
Amoeba—fresh	0.70	41	2.50
Stained—hematoxylin	4.50	27	15.00
Rose bengal test	8.20	49	25.00
Hippuric acid excretion	1.83	11	5.00
Basal metabolism (technical only)	2.80	17	10.00
Electrocardiogram (technical only)	5.20	31	15.00
Autopsy (technician time only)	10.40	62	
Autopsy (including pathologist's time)	26.40	158	
Surgical tissue (technical only)	0.95	6	
Surgical tissue (including pathologist's time)	2.30	14	

from patients. Table 2 illustrates, for a few common tests, the detailed method of study applied to arrive at the total cost of each test.

Table 3 lists the common clinical laboratory procedures by total cost in cents. It is not supposed that this will apply exactly to other laboratories with different salaries and space values. The ratio between the various tests should be relatively constant however, and make such costs easy to figure anywhere. If the cost of a routine urinalysis—one-sixth of a dollar here—be taken as a unit, the second column, table 3, shows the other tests' relative cost in such "urinalysis units."

In this hospital charges for most tests are adjusted to from three to five times the technical cost of the test. These charges are tabulated in column 3 of the table 3. The margin thus provided pays for uncollected charges; for the cost of autopsies and routine surgical tissue examinations for which no charges are made; for laboratory measures taken to safeguard health in the hospital and to prevent epidemics, such as nose, throat and stool cultures of nurses and food handlers, water and milk examinations etc.; and for the time required for teaching internes, residents and nurses. The cost, unexpectedly high, of these "good will," unremunerated procedures is shown in table 4.

TABLE 4

"Good Will" Work (Uncharged)

To maintain A. M. A. and A. C. S. rating and public health of hospital

PROCEDURE	UNIT COST	TOTAL
Autopsies—100—46 per cent deaths	\$26.45	\$2645.00
Surgical tissue—gross examination only—1,138	0.64	728.00
Surgical tissue—gross and microscopic—1,028	2.05	2107.00
Laboratory work on employees		1000.00
Total		\$6,480.00

SUMMARY

Laboratory costs in an average sized general hospital are analyzed into their component factors. Totals of each of these are given for the year to establish the ratio between them. The cost of each individual test is then determined and expressed both in dollars and cents and in terms of "urinalysis units"—i.e. the cost of a routine simple urinalysis. A schedule of charges, based on three to five times the technical cost, is suggested. Finally, the cost of free work, done to maintain the hospitals' health and rating, is analyzed.

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EDITORIAL

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THE CASE FOR THE QUANTITATIVE WASSERMANN REPORT

Among the recommendations of The Committee On The Evaluation of Serodiagnostic Tests For Syphilis—which, it can be said with certainty, they do not promulgate as ex cathedra pronouncements—was that the results of such tests be reported simply as "positive," "negative," and "doubtful."

While there is little question that such a method of reporting is so simple and definitive that "he who runs may read," it seems open to discussion whether this method does not represent a backward rather than a forward step in the campaign against syphilis.

Certainly the prime purpose of the campaign is not merely the *detection* of syphilis. If this were true, then quite obviously a simple determination of the presence or absence of syphilitic reagin would suffice. But the underlying purpose is the eradication of syphilis the first step toward which is, of course, the recognition of its presence, particularly in the infective stages of the disease.

Now, whether so expressed or not, the patient confronted with a diagnosis of syphilis, at once desires to know how much treatment and how long a period of time will be required to bring the disease under control.

The conscientious practitioner, no matter how great his skill—or, better, the greater his skill and training—is forced to admit inability to answer this question with certitude. Too many factors are concerned: the duration and virulence of the infection; the degree to which it has obtained systemic lodgement; the ability of the patient to absorb and respond to the effects of drugs, and so on. Nevertheless, some answer must be attempted in general terms in the estimate of which the quantitative serologic report has been and can be of definite value.

It is no longer necessary to labor the point that two serums may be "positive" or "plus four" and still vary widely in their reagin content. So widely, in fact, that though treatment may have produced a definite decrease in the reagin content, as shown by a quantitative technic, the serum may still be "plus four" by a qualitative method.

Obviously, it seems fairly reasonable to assume that a patient whose blood is only weakly positive may not require as intensive or prolonged treatment to become seronegative—and, hence, with his disease under control—as one whose blood evidences a high reagin content. And as, without a quantitative report, such a difference is not evident, to that extent a quantitative report is of definite value to the physician.

Is a quantitative report of any use to the patient?

Probably the most important factor conducive to success in the treatment of syphilis is persistence. In other words, treatment must not only be adequate in amount, but continued over a sufficiently extended time. And to achieve this the entire cooperation of the patient and his persistence in seeking treatment is of essential and obvious importance. In order to secure this coöperation—and this is especially true in private practice—the patient must not be led to expect too much or too little; he must be neither too optimistic nor too despondent as to the results to be expected, for there is an element of psychology in the treatment of syphilis which must not be overlooked.

Consider two patients, each with a "positive" reaction. One, by a quantitative technic shows a 40000 reaction, the other 44440. Each is in the hands of a different physician, each receives the same amount of treatment over a stated period of time at the end of which the first patient may have a 20000 reaction or even a clear-cut negative. The other, though quantitatively reduced to 44000 still has a "positive" reaction.

As it appears to the patient, the first has had a definite result from treatment; the second shows no apparent change—still "positive."

Unless such a patient is particularly intelligent and the subject has been discussed with him in great detail, one of two

things is almost inevitable. He becomes discouraged, despondent, and skeptical as to the results to be expected and tends to drift into the hands of the quack, the faddist, the crook or the cult; or, what is worse in its effect on the reputation of his physician, he loses faith in him and his methods and goes elsewhere—quite naturally to him whose treatment produced "negative" results in the same time.

Quantitative technics are neither so technically difficult, so laborious or so time-consuming as to warrant elimination on that score—even were such objections cogent, which they are not.

It may be freely granted that quantitative methods in serology are not as accurate as measurements by weight or volume. But they do establish variations in reagin content and so furnish evidence of clinical value and utility.

The value of the quantitative serologic report is further discussed at some length in another place in this issue.

R. A. KILDUFFE.

NEWS AND NOTICES

At the Second Annual Meeting of the New York State Society of Pathologists the resolutions following were adopted. Because of their interest to pathologists in general, they are given in full below:

I. Whereas the present position of Pathologists and Laboratory Directors in New York State should be improved, and it is desired that changes in the relationship between Hospital and Pathologist be, in some cases instituted, and Whereas it is difficult to outline a set single policy in all cases, applicable to all locations:

Therefore Be It Resolved that certain general principles apply in any such contractual relationship particularly

1. Inasmuch as the practice of Pathology constitutes the practice of a recognized specialty in Medicine, therefore every Pathologist should be a member of the Medical Board of the Hospital, with vote,

2. That such contracts include definite duties and obligations of the Pathologists and Directors, and in no case exclude the right of the individual Pathologist to private consultations, entirely exclusive of his contractual obligations to the Hospital, and that this be true whether the Pathologist be employed on a contract full time or part time basis or individual fee schedule; and

3. This Committee urges that members of the Society agree to the understanding of applying ample, ethical, professional standards in our relationship to other Pathologists, and to refer all grievances and difficulties arising from this source (covering vacancies in hospitals) to this Organization.

4. Furthermore, Be It Resolved that this Committee make a further study of such contractual relationship in general, and recommend additional principles as a basis for such contracts and approved satisfactory contract forms.

II. Whereas Pathology is a definite specialty in the practice of Medicine, this Committee recommends definite disapproval of the practice of Pathology on a full time, salaried basis with the status of employee only, and on the principle that pathological work be considered the practice of Medicine by the Individual,

Therefore, Be It Resolved that

1. In keeping with the above, all technical work and reports come directly under the Laboratory Director's responsibility, and all technical personnel be considered as working under his supervision; and

2. We Further Recommend that in Hospital Plan Insurance cases and com-

pensation cases the Pathologist be credited with a definite percentage of a day's fee in proportion to the work done.

 We Further Recommend, strongly favor and urge the elevation of the present status and standards and salary scale of all supervised technical personnel.

III. Finally, as an expression of our sentiments and to favor coöperation, we urge that a copy of these resolutions be sent to the American Medical Association and the American College of Surgeons, the American Hospital Association, The New York State Society, to the individual County Medical Societies, and to the Society of Roentgenologists and Anesthetists.

IV. Whereas the Department of Laboratories of the New York State Board of Health having done excellent work in raising the standards of service in the laboratories subsidized by the State, and in this way have realized all the

advantages that State support may properly render.

Be It Resolved that this Committee urges that the State Department of Health henceforth adopt the policy of gradually withdrawing State support of such Laboratories, reducing the costs of those laboratories to the taxpayers, and limiting their services to communicable diseases, and to those who are indigent. That all other services rendered by these institutions should be on a fee basis.

Resolved: that (1) It is contrary to the purpose of the New York State Society of Pathologists for any physician to be employed by or to permit his name to be used by any private or commercial laboratory which is not in actual fact owned and operated by a fully qualified pathologist who is eligible to membership in this Society.

(2) Any physician accepting employment or permitting his name to be used by a laboratory other than above, shall not be eligible for election to membership nor to retain membership in the New York State Society of Pathologists.

(3) A laboratory shall be considered a commercial enterprise whenever a non-medical person participates and shares in the profit of the operation of such an institution is not eligible for membership in this Society, except that the Council may approve professional affiliation and/or partnership between a qualified, accredited pathologist and other qualified experts in affiliated sciences.

(4) Furthermore, no member of this Society who operates a laboratory shall be permitted to retain his membership in the Society if he allows rebating of

fees under any guise whatsoever.

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Announcement is made of the appointment of the following Committee on Code of Ethics:

DR. HENRY F. HUNT, Chairman

DR. O. B. HUNTER

DR. T. D. ROBERTSON

DR. Z. BOLIN

DR. A. S. GIORDANO

Dr. S. H. Curtis

The Roster: The following information pertaining to the Roster has been received from Dr. Giordano: Counselor for Connecticut, Dr. J. W. Fisher; Counselor for Minnesota, Dr. Charles R. Drake.

The following preliminary program outline has been issued for the first American Congress on Obstetrics and Gynecology to be held at Cleveland, Ohio, on September 11–15, 1939:

PRELIMINARY PROGRAM OUTLINE

Medical Section

Monday, September 11, 1939: The Thyroid and Pregnancy; Heart Disease and Pregnancy; Diabetes and Pregnancy; Tuberculosis and Pregnancy; Nutritional Factors and Pregnancy; The Surgical Abdomen Complicated by Pregnancy; The Treatment of Abortions.

Tuesday, September 12, 1939: The New Conception of Ovarian Neoplasms; Carcinoma of the Uterus; Endometriosis; Ectopic Pregnancy; Sterility in the Female.

Wednesday, September 13, 1939: Reduction of the Operative Incidence in Obstetrics; Labor Complicated by the Contracted Pelvis; Dystocia Due to Soft Parts; Pathology and Treatment of the Third Stage of Labor.

Thursday, September 14, 1939: Present Day Fundamental Knowledge of Hormones and Endocrine Glands; Problems of Adolescence; Problems of Menopause; Diseases of the Mammary Gland.

Friday, September 15, 1939: Sulfanilamide in Obstetrics and Gynecology; Pyelitis; Chronic Pelvic Infections; Immediate and Remote Complications Following Labor.

Round Tables

Running concurrently each day 11:45 to 1:15: The Toxemias of Pregnancy; Genital Infections; Obstetrics and Gynecologic Hemorrhages; The Fetus and the Newborn; Forceps, Occiput-Posterior, and Breech Presentation; Anesthesia, Analgesia and Amnesia in Labor.

Joint Afternoon Sessions

Monday, September 11, 1939: Neonatal Care.

Tuesday, September 12, 1939: Plans For Prevention and Control of Uterine Cancer.

Wednesday, September 13, 1939: Extension Education on Maternal and Neonatal Care.

Thursday, September 14, 1939: Economic Aspects of Maternal Care.

Friday, September 15, 1939: Correlation Of and Attempt to Digest All Proceedings.

Joint Evening Sessions

Monday, September 11, 1939: Legal Aspects of Maternity. Tuesday, September 12, 1939: Humanitarian Aspects. Wednesday, September 13, 1939: Sociologic Aspects. Thursday, September 14, 1939: Ethical Aspects.

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Announcement is made of a change in name of the official publication of the Mississippi Valley Medical Society. Heretofore known as the Radiologic Review and Mississippi Valley Medical Journal, the new publication will be known as The Mississippi Valley Medical Journal (Incorporating The Radiologic Review). Under the Editorship of Dr. Harold Swanberg, Quincy, Illinois, the new Journal will be issued bimonthly and will be devoted to clinical medicine, surgery and radiology. There is no change in the Editorial Board composed of clinicians and radiologists.

Dr. W. S. Thomas has left the Clifton Springs, New York, Sanitarium to become Director of The Monroe County Laboratory and Pathologist to the Monroe County Hospital at Rochester, New York.

His new address is 435 East Henrietta Road, Rochester, New York.

The 68th Annual Meeting of the American Public Health Association will be held in Pittsburgh, Pa., October 17–20, 1939, with headquarters at the William Penn Hotel. The Chairman of the Local Committee will be Dr. I. Hope Alexander, Director of Health of Pittsburgh.

ERRATUM

Dr. F. W. Hartman calls attention to two errors in his paper "Some Etiological Factors And Lesions In Cerebral Anoxemia" which appeared in the November issue.

On page 636, in the third paragraph referring to the use of nitrous oxide in obstetrics, the concentration should be 90 per cent instead of as given.

In the second line from the bottom of the page, the dosage of nembutal should be 3 grains.

BOOK REVIEWS

Diseases of The Skin For Practitioners and Students. By George Clinton Andrews, A.B., M.D., Associate Professor of Dermatology, College of Physicians and Surgeons, Columbia University; Chief of Clinic, Department of Dermatology, Vanderbilt Clinic; etc. Ed. 2. Entirely reset. 899 pp., 938 illustrations, \$10.00. W. B. Saunders Co., Phila., Pa. & London.

This is an excellent book deserving of a place in the reference library of every physician.

This edition has been brought up to date by the addition of over seventy-five new diseases, new chapters on diseases due to filterable viruses, vitamin deficiencies and cutaneous infiltrations with metabolites and extensive additions and revisions on almost every page. The book is well and clearly written, well organized and bears ample evidence of wide experience and wide familiarity with the literature. The illustrations are excellent and numerous.

All in all this is an excellent and practical text.

Textbook of Bacteriology. By Thurman B. Rice, M.D., Professor of Bacteriology and Public Health, Indiana University School of Medicine. Ed. 2, Cloth, 563 pp., 121 illustrations. \$5.00. W. B. Saunders Co., Philadelphia.

It is not surprising that this book has reached a second edition within a

relatively short time, for it is an excellent and practical text.

Intended primarily for the student, this book may well be read by the physician for its clear and simple discussions. Technical procedures described are limited to those feasible with a minimum of equipment and skill. The securing of samples for more elaborate examinations is described in detail.

This book may be commended as a clear, readily understandable and rela-

tively simple exposition of an important subject.

Textbook of Bacteriology. By Edwin O. Jordan, Ph.D., Late Andrew McLeish, Distinguished Service Professor of Bacteriology, University of Chicago. Revised by William Burrows Ph.D., Assistant Professor of Bacteriology, University of Chicago. Ed. 12, Cloth, 808 pp., 197 illustrations. \$6.00. W. B. Saunders Co., Philadelphia.

Professor Jordan's "Bacteriology" is a book requiring no introduction for it has long been a favored reference text for laboratory workers, physicians and

students alike.

The present (12th) edition has been extensively revised to embody the rapid and numerous advances since the last edition in 1935.

As heretofore, this book can be highly recommended as an excellent and comprehensive reference and working text which, indeed, requires neither introduction nor recommendation to pathologist or physician.

Pathological Anatomy and Pathogenesis. By Horst Oertel, Strathcona Professor of Pathology, Director of the Pathological Institute, The McGill University, and Pathologist-in-Chief to The Royal Victoria Hospital, Montreal, Canada. Cloth, 640 pp. Renouf Publishing Co., Montreal, Canada. This book is the outcome not only of the author's lectures to students but of his firm conviction that, as quoted in the Preface, "pathological anatomy is the conscience of the physician."

Its purpose is to lead the student—and the physician—to look upon diseases

primarily as living tissue movements.

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While this volume is restricted to the special pathological anatomy of the circulatory, respiratory, renal and digestive systems, it embraces indirectly a wealth of information. Scholarly, well written and with a sane and logical viewpoint, this book may well be read with profit and interest by the physician and pathologist. It is, indeed, a stimulating book.

A B C of Vitamins, A Survey In Charts. By Jennie Gregory, M.S. With a Foreword by Walter H. Eddy, Professor of Physiological Chemistry, Teachers' College, Columbia University. Cloth, 93 pages, 56 charts. \$3.00. Williams & Wilkins Co., Baltimore, Md.

This is not only a most interesting but an exceedingly informative book, a companion volume to the author's previous "A B C of The Endocrines."

By means of cleverly constructed charts and graphic drawings on the style of "pictorial statistics" the author presents a world of information concerning the vitamins.

While readily intelligible to the layman to whom, perhaps, it is primarily addressed, this book is also of value to the physician, physiologist, pathologist and, indeed, to all who may be seeking the story of the vitamins in a nutshell, as it were—for this book is indeed a multum in parvo.

It can be recommended without reserve as presenting all that is at present known of the vitamins in an exceedingly assimilable fashion.

The Pituitary Gland. An Investigation of The Most Recent Advances. Cloth, 764 pp., 160 illustrations, 6 plates. \$10.00. The Williams & Wilkins Co., Baltimore, Md.

This is Volume XVII of a series of Research Publications issued by The Association for Research In Nervous & Mental Disease, edited by Walter Timme, Angus M. Frantz and Clarence C. Hare.

The book is divided into three sections. Section 1 contains five papers covering various aspects and investigations of the anatomy of the pituitary gland; Section 2 contains 22 papers discussing investigations of the physiology of the pituitary; and Section 3 contains 15 papers which, under the heading of General Considerations, discuss a variety of subjects related to pituitary disorders.

A list of members of the Association and an index complete the volume. Those interested in the functions and dysfunctions of the pituitary will find

this volume not only interesting but stimulating.

The Chemistry of the Sterids. By Harry Sabotka, Chemist to the Mt. Sinai Hospital, New York City. Cloth, 336 pp. \$8.50. The Williams & Wilkins Co., Baltimore, Md.

This book, a companion volume to the author's previous book on the Physiological Chemistry of The Bile, presents probably the most complete and comprehensive discussion of the bile acids and sterols thus far available.

As the author comments in his preface, despite the voluminous publications relative to the chemical, biological and specific properties of the bile salts and acids, these investigations have not heretofore been systematically correlated. Moreover, as an aftermath of recent chemical investigations there has been a relatively enormous increase in the number of known bile acids and sterol derivatives, a systematic account and correlation of which are the subject of this book. A special chapter is devoted to analytical methods.

In addition, he presents for the first time a classified catalogue of sterids and their derivatives recorded before January 1, 1937.

This is a volume sui generis which, as a reference text, is not to be displaced.

Manual of Psychiatry and Mental Hygiene. By Aaron J. Rosanoff, M.D. Cloth, Ed. 7, 1091 pp.; 85 figures, 1 colored plate. \$7.50. John Wiley & Sons, Inc., New York.

In the author's words, the purpose of this book is "to give a comprehensive bird's-eye view of the entire field of psychiatry and mental hygiene." That he has not failed in this objective is evidenced, not only by the fact that the book has reached a seventh edition, but by the book itself which bears ample evidence of extensive and well digested experience.

The present edition has been thoroughly revised and extensively rewritten to bring it in line with the many advances and developments in this field which have taken place since the last edition, eleven years ago.

Part I (194 pages) is devoted to a discussion of General Psychiatry; Part II (468 pages) to Special Psychiatry; Part III (178 pages) presents a discussion of the Practice of Psychiatry; Part IV (71 pages) discusses Mental Hygiene; and Part V (144 pages) details Special Diagnostic Procedures. Part VI contains as appendices normal tables and various classifications. There is an extensive glossary and author and subject index.

This book can be highly recommended as an earnest, thoughtful, comprehensive and authoritative discussion. As a working manual it stands in a class by itself.

Glaister's Medical Jurisprudence and Toxicology. Edited by John Glaister, M.D., D.S.C., Barrister-at-Law, Regius Professor of Forensic Medicine, University of Glasgow; Formerly Professor of Forensic Medicine, University of Egypt, Cairo; and Medico-Legal Consultant To The Egyptian Government. Cloth, Ed. 6, 747 pp.; 107 illustrations and 8 plates. \$8. William Wood & Co., Baltimore Md.

This well-known work achieved status as an authoritative and comprehensive text with the appearance of the first edition and each subsequent edition has added to its standing.

This book needs neither introduction nor specific commendation; it speaks for itself.

The present edition is improved in many respects. Many of the small type interpolations of previous editions have been eliminated in favor of larger type as a whole; the index is more comprehensive; the subject matter has been largely reorganized and added to; and there are numerous new illustrations.

Among the new additions are discussions of dermal prints, palmar prints, identification of maggots, grouping of blood and seminal fluid, technic for sectioning and identification of hairs, uses of filtered ultra-violet light, and war gases. Among the more striking additions to the text is the inclusion of a resume of the problems of identification in the Ruxton case which has already been published separately in full detail (The Medicolegal Aspects of the Ruxton Case—Glaister and Brash).

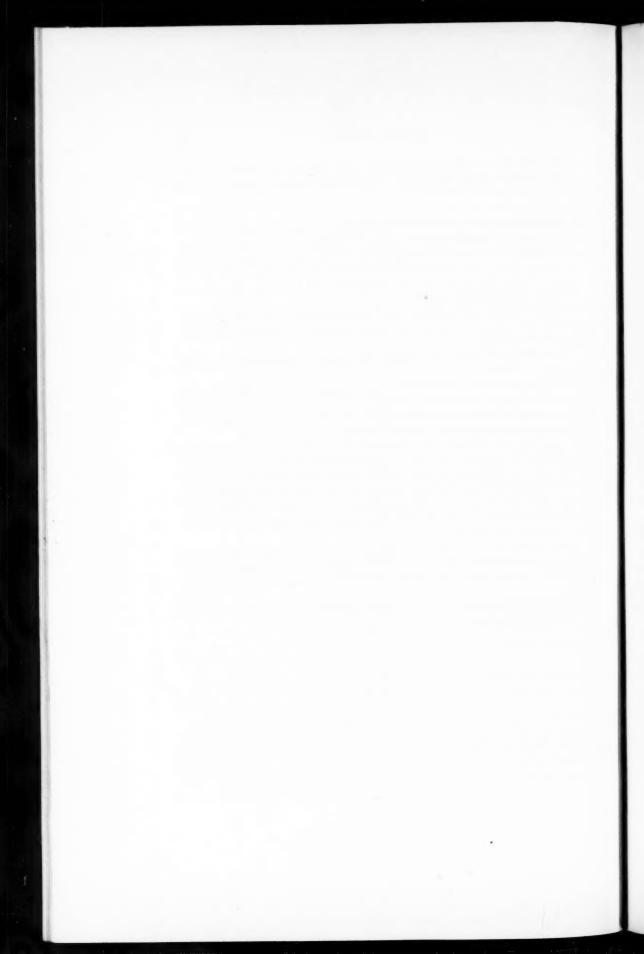
While based largely upon European methods and aspects of medicolegal problems, this standard volume is so comprehensive and so authoritative as to deserve an honored place in the library of all those to whom such problems may come.

The lawyer, the pathologist and even the physician at large may consult it with interest and profit.

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A CRITIQUE OF CHEMICAL PROCEDURES USED IN CLINICAL DIAGNOSIS*

M. BODANSKY

John Sealy Hospital, Galveston, Texas

The third edition of Todd's "Clinical Diagnosis," published in 1919 contains a paragraph on the "chemic examination" of the blood in the section entitled "Less Frequently Used Methods." It is prefaced by the remark: "In this section brief consideration will be given a number of methods which are not as yet in common use, some because their clinical value has not been proved, others because the technic has not been sufficiently simplified." In the paragraph reference is made to the Lewis-Benedict method for blood sugar; the picric acid method for creatinine; and Folin's new direct nesslerization methods for urea, non-protein nitrogen and total nitrogen.

I refer to this classic, first out of respect and profound admiration for its author, our late friend and colleague, and second because, in the 28 years of its existence, this book has so faithfully reflected in its successive revisions the onward progress of clinical pathology in its most practical aspects. The sixteen lines which two decades ago alluded to the methods of blood chemistry have expanded into a chapter of nearly sixty pages, under the caption "Clinical Chemistry."

In accepting this opportunity to discuss the subject, I wish to do so, not as a biochemist, but as a clinical pathologist, and as such I propose to devote myself primarily to those procedures which are pertinent to every-day medical practice. Moreover, I shall concern myself principally with questions of technical precision, for it must be evident that a wrong analytical result is seldom, if ever, productive of a correct clinical interpretation.

^{*} Received for publication July 5, 1938.

Read before the Seventeenth Annual Meeting of The American Society of Clinical Pathologists, San Francisco, California, June 9-11, 1938.

The term "clinical chemistry" has come to be used almost synonymously with "blood chemistry," largely because the rapid development in the technic of blood analysis and the advances in the clinical interpretation of the data have overshadowed other chemical procedures. Other body fluids, as well as the dejecta, receive today approximately the same qualitative treatment as they did a generation ago. The development of a more quantitative viewpoint toward the changes in the composition of cerebrospinal fluid, transudates, exudates and other biological materials may contribute materially to the comprehension of disease as it affects the individual patient.

With these considerations in mind, we may nevertheless turn our attention first to the more commonly used methods of blood analysis. Obviously, it will be impossible to dwell at length either on technical details or on points of clinical interpretation.

PROTEIN

The determination of serum (or plasma) protein finds its greatest application in relation to edema. Loss of protein by excretion (nephrosis, nephritis), malnutrition and failure in regeneration (hypogenesis due to hepatic involvement) are the three important factors which result in depletion of the plasma proteins. It may be observed that even in edema of cardiac origin, the reduction in blood protein due to inadequate protein intake is often an important factor. The edema which occasionally accompanies rapid blood regeneration in patients with anemia under specific treatment is fundamentally a nutritional type, being related to the withdrawal of protein from the blood and tissues for the purpose of hemoglobin synthesis.

Since the osmotic properties of serum albumin and serum globulin differ so widely, the determination of both fractions is found important. Yet there is a strong probability that these fractions may have no real being in the plasma itself. McFarlane¹ has shown that the fractionation of the plasma proteins by ultracentrifugal sedimentation yields quantitatively different results from those obtained by precipitation. Higher results are obtained for albumin and lower results for globulin than are found by chemical analysis, which may mean that in the salting-out

preliminary to analysis changes are produced which alter the serum proteins, as regards their molecular size and possibly in other respects as well. It is also significant that when serum is diluted the fall in colloid osmotic pressure is correspondingly greater than the fall in concentration, indicating an increase in the average molecular size of the protein on dilution. It has been suggested (Block)² that there exist in serum two principal co-precipitation systems which are interdependent, yet possess a sufficient degree of independence to permit their separation one from another.

From the foregoing considerations it is to be realized that although the determination of serum proteins is of considerable diagnostic value it is nevertheless essentially an empirical procedure.

Serum or plasma protein analysis consists of the following determinations:

(1) Total nitrogen of serum (or plasma).

(2) Total nitrogen of globulin free filtrate. Globulin is precipitated in a 1.5 Molar concentration of sodium sulfate (Howe's method).

(3) Non-protein nitrogen of serum (or plasma).

The total protein is represented by (1) - (3); the albumin by (2) - (3) and the globulin by (1) - (2). The globulin may also be obtained by subtracting the albumin from the total protein.

Equations have been developed for calculating the colloid osmotic pressure from the analytical data for serum albumin and globulin. According to Wies and Peters³ the relation is best represented by the equation:

$$\pi = 60.9 \times A_w + 22.9 \times G_w - 50$$

Where π is the osmotic pressure in millimeters of water; A_w the grams of albumin and G_w the grams of globulin, per kilo of water. The concentration of serum protein and its fractions may be corrected to grams of protein per kilo of water by means of the equation W=98.4-0.718 P, where W is the amount of water and P the amount of protein (Eisenman, MacKenzie and Peters)⁴.

NON-PROTEIN NITROGEN

The non-protein nitrogen is most conveniently determined by micro-Kjeldahl digestion (Koch-McMeekin method).⁵

The total N and the nitrogen of the globulin-free filtrate may be determined by one of the following methods:

(1) Kjeldahl method.

(2) Micro-Kjeldahl (Koch-McMeekin method).

(3) Colorimetric method.

The Kjeldahl method is usually the most reliable, but satisfactory results may be obtained also by the micro-Kjeldahl technic, as improved by the Koch-McMeekin method. colorimetric method depends on the chromogenic property of protein when treated with Folin's phenol reagent, or one of its modifications (Folin-Ciocalteau reagent). A similar color is given by tyrosine, a solution of which is used as the standard. It remains to be proven that in disease (nephrosis, etc.) the proteins are unchanged as regards their chromogenic property. is perhaps significant that the colorimetric method since its publication by Wu⁶ in 1922 has undergone many modifications (Greenberg⁷, Minot and Keller⁸, Andersch and Gibson⁹, etc.). A recent modification published in this Journal is that of Johnston and Gibson¹⁰. The colorimetric method is applicable to the determination of cerebrospinal fluid protein.

In our hands, the Koch-McMeekin micro-Kjeldahl procedure has been found most practical. For digestion of the Folin-Wu filtrate 1:1 sulfuric acid is recommended instead of the phosphoric-sulfuric acid mixture used in the original Folin-Wu technic. Digestion is brought to completion by the addition of a drop of 30 per cent H₂O₂ (Merck's blue label Superoxol). The nitrogen is determined by direct nesslerization (Folin's Nessler's reagent, as modified by Koch and McMeekin)⁵.

UREA

Reliable results may be obtained by the Van Slyke-Cullen urease and aeration procedure, as well as by the well known methods of Folin and Wu and Folin and Svedberg. However other procedures requiring less manipulative skill are better adapted to the clinical laboratory. The urea in the Folin-Wu filtrate may be hydrolyzed and the resulting ammonia determined by direct nesslerization (Karr's method). Another procedure is to first treat the blood with urease, then precipitate the protein and nesslerize the filtrate. Koch recommends the use of ordinary Folin-Wu blood filtrate in the preparation of the standards in order to avoid differences in tint. In the Leiboff-Kahn¹² method the urea (Folin-Wu filtrate) is hydrolyzed in the presence of acid

under pressure. The temperature is raised to 150° and maintained at this point for 10 minutes. In our experience more consistent results are obtained if the temperature is not raised above 140°C. The ammonia formed is determined by direct nesslerization.

It is advisable to determine both urea or non-protein nitrogen. One analysis serves as a check on the other and occasionally a low urea accompanying a normal or high non-protein furnishes evidence of severe impairment of hepatic function.

In the urea clearance test determination of urine urea is required. This may be done by the Van Slyke-Cullen method or by one of a number of direct nesslerization procedures (Folin and Youngburg, Koch and McMeekin, etc.). The hydrolysis method of Leiboff and Kahn has also been adapted to this purpose. In these analyses the ammonia nitrogen is either determined separately and the amount subtracted from the urea + ammonia nitrogen, or else the ammonia is first removed by permutit.

However these steps may be omitted since the ratio

Urine Urea N + Ammonia N Blood Urea N

may be used in the equation in preference to the ratio

Urine Urea N Blood Urea N

Indeed a simplified procedure has been proposed by Van Slyke and Cope¹³ in which no standards are required. The urea of both blood and urine is converted into ammonia with urease; protein and other interfering substances are removed and the ammonia contents of both filtrates, after proper dilution and nesslerization, are compared with each other.

AMINO ACID NITROGEN

The determination of amino acids in blood though not frequently requested may be of occasional value in demonstrating failure of hepatic function such as occurs in acute yellow atrophy. A marked rise in the amino acid concentration above the usual range of variation (4–8 mgm.), at the expense of urea is significant.

The colorimetric method of Folin¹⁴ is rapid, though perhaps

somewhat less specific than the nitrous acid method of Van Slyke. Folin's procedure is based on the color reaction produced with the sodium salt of β naphthoquinone-sulfonic acid. The standard solution contains glycine and glutamic acid.

URIC ACID

Folin's direct colorimetric method depends on the reduction of phosphotungstic acid. The reaction is not altogether specific. Ergothioneine and glutathione liberated from the corpuscles through hemolysis give the reaction. This may be obviated by the use of unlaked blood filtrate, as recommended by Folin. However for most practical purposes this is not essential.

The method of Benedict and Behre¹⁵ is based on the reduction of arsenotungstic acid. The color comparison may be made when the solutions are still warm, thereby avoiding the difficulty due to development of turbidity.

CREATININE

With careful attention to details the method of Folin and Wu gives dependable results, although the change in the color of the picrate solution produced by amounts of creatinine normally present in blood is unimpressive. However an increase above normal is readily detected and may be determined with reasonable accuracy.

A second procedure, developed independently by Langley and Evans¹⁶, Benedict and Behre¹⁷ and Bolliger¹⁸ is based on the reduction of the sodium salt of 3.5 dinitrobenzoic acid. The method may also be applied to the estimation of creatine (Andes)¹⁹. Some find it easier to compare the color obtained with this reagent than that produced with alkaline picrate.

The significance of this determination has been enhanced by identification of the chromogenic substance as creatinine. For a long time this has been a subject of controversy and certain investigators even doubted that any part of the color produced was actually due to creatinine. However Miller and Dubos have shown that the chromogenic substance in human blood is decomposed by two bacterial enzymes each possessing a high

degree of specificity for creatinine. This seems to be incontrovertible evidence that creatinine constitutes most, if not all of the chromogenic (Jaffe-reactive) material in normal blood.

GLUCOSE

A fundamental shortcoming of the older procedures was that the effect of non-sugar reducing substances was not excluded in the blood sugar determination. The results obtained by the original method of Folin and Wu, which is still widely used, yields values which are approximately 10–15 per cent higher than the true glucose concentration. The use of unlaked blood filtrate in Folin's improved method corrects this error. In our hands, the Benedict modification²⁰ has been found satisfactory. Benedict's copper reagent contains alanine in place of tartrate and the reaction is made more specific for glucose by the addition of a drop of bisulfite solution. The Folin-Wu filtrate may be used, as well as the Folin-Wu sugar tubes.

Other modifications of the original Folin-Wu method may be found in the literature. Among the procedures based on somewhat different principles is the Shaffer-Hartman titration method and its modification by Somogyi, the ceric sulfate microtitration method of Miller and Van Slyke and the well-known procedure of Hagedorn and Jensen. Each of these present some particular advantage especially for those who prefer titrimetric to colorimetric methods of analyses. However from the standpoint of accuracy and adaptability to routine laboratory work the Benedict modification of the Folin-Wu method fulfills most requirements.

A number of micro-analytical sugar methods requiring as little as 0.1 cc. of blood have been devised. Byrd's method is essentially an adaptation of the Folin-Wu procedure. The blood is hemolyzed and precipitated in a diluting pipette. The filtrate is heated with the copper reagents in a tube similar to the Folin-Wu sugar tube, only smaller. The method of Folinand Malmros depends on the reduction of ferricyanide by the glucose, the conversion of the ferrocyanide thus formed into Prussian blue, and comparison with an appropriate standard.

CHOLESTEROL

Bloor's colorimetric method in its original form, or as modified by Sackett and others has maintained its popularity as a clinical procedure.

Schönheimer and Sperry's method depends on extraction with a mixture of acetone and absolute alcohol. The cholesterol is precipitated as the digitonide, separated by centrifuging, dried, washed with an acetone-ether mixture, dissolved in acetic acid and treated with acetic anhydride and concentrated sulfuric acid. The color thus developed is compared with appropriate standards. A detailed description of the method with certain improvements as applied to total and free cholesterol has been published in this Journal by Sperry²¹.

A system of differential lipid analysis has been recently summarized in this Journal by Boyd²².

CARBON DIOXIDE COMBINING CAPACITY (ALKALI RESERVE)

The Van Slyke-Cullen method for CO₂ combining capacity fulfils most requirements, although some prefer the manometric method of Van Slyke and Neill. The older procedure is, however, more adaptable to most laboratories where the determination is done at infrequent intervals.

The alkali reserve may also be determined by the Haskins-Osgood modification of the Van Slyke-Stillman-Cullen titration method.

CHLORIDES

Chlorides may be determined on the Folin-Wu filtrate by Whitehorn's method. This is based on precipitation by an excess of standard silver nitrate and back titration with sulfocyanate in the presence of ferric alum as indicator.

Whole blood, serum or plasma may be analyzed directly by the method of Van Slyke or by the improved procedure of Wilson and Ball. The blood serum or plasma is first digested with concentrated nitric acid and an excess of standard silver nitrate on the water bath, and the excess silver nitrate subsequently determined by titration with sulfocyanate.

For analysis of plasma or serum, the blood should be collected under mineral oil.

CALCIUM

In our hands the Clark-Collip modification has been found very dependable. However, Koch⁵ first liberates the ionic calcium with 20 per cent trichloracetic acid, at the same time precipitating the protein. The calcium in the filtrate is converted into oxalate, washed, dissolved in sulfuric acid, and the heated solution is titrated with permanganate.

Larson and Greenberg²³ have published an oxidimetric titration method which is claimed to be superior to the permanganate titration. The calcium, precipitated as oxalate, is separated by filtration on a Kirk-Schmidt microfilter. It is then dissolved in hot sulfuric acid and titrated with 0.01 ceric sulfate, using o-phenanthroline as indicator.

Roe and Kahn have devised a colorimetric method based on the precipitation of the calcium as the triphosphate and colorimetric determination of the phosphorus in the precipitate.

PHOSPHORUS

The Fiske-Subbarrow method depends on the formation of ammonium phosphomolybdate, which is in turn reduced by 1,2,4-aminonaphthol sulforic acid.

Reduction of phosphomolybdate by stannous chloride is the basis of a number of methods (Kuttner and Lichtenstein, Youngburg and Youngburg, and A. Bodansky).

PHOSPHATASE

This enzyme liberates phosphate from organic combination; serum (or plasma) is incubated with sodium β -glycerophosphate for a given period. Depending on the phosphatase activity, a larger or smaller amount of phosphate is liberated. The determination has been found to be of value especially in bone and liver disease. The more widely used procedures are those of Kay²⁴, Roberts²⁵ and A. Bodansky²⁶.

I have doubtless fallen short of accomplishing my assignment, which from the title implies a critical, careful and thorough analysis of the subject. In defense I may say that I have avoided those procedures, now more or less abandoned, which would have afforded more of an opportunity for fault-finding. To have selected such methods intentionally would have been like seeking open doors to break through, for as I have indicated there is at least one practical and satisfactory method for every important blood constitutent—and this statement may also be applied to gastric analysis and other phases of chemical pathological technic.

In the evaluation of any technical procedure there are three pertinent questions to be answered: (1) is the method specific for the substance to be determined? (2) does it give a true measure of the amount present? (3) is it possible to further simplify the technic, without diminishing the degree of accuracy?

For a few procedures the first two questions may be answered affirmatively and it might perhaps be added that further simplification is of no immediate importance. With other procedures, fault could be found on one or more counts, but despite certain deficiencies, the methods that have been outlined yield acceptable results according to prevailing standards.

Chemical analysis can be simplified just so far and no further. The procedures I have indicated properly belong within the province of the clinical pathologist and the clinical pathological laboratory. Such work cannot be expected to come out of the pretty little wooden boxes, containing nicely labeled sets of reagent bottles which seem to acquire increasing popularity. The clinical pathologist need not be admonished on this score, but it is important that the physician is made aware of the inherent limitations of any but standard procedures, performed in an adequately equipped laboratory supervised by a competent individual. For one of two semi-quantitative procedures, these "outfits" may have some value, but to expect useful results for urea, uric acid, creatinine, calcium etc., from any such make-believe, toy-laboratories is nothing short of folly. As small children we were intrigued by chemical sets; it was fun to mix the contents of bottle no. 1 with the contents of bottle no. 2 and to watch the smoke, or the change in color. But this idea of childhood play should hardly be carried over into the serious field of clinical diagnosis. Neither chemical analysis, serology, nor any other kinds of clinical pathological procedure that I know of have attained such a degree of simplicity as to make them automatic. Clinical pathological technic still requires a measure of skill, as well as a cultivated sense of awareness which makes possible the detection of error, and moreover no result can have any meaning without a comprehensive interpretation.

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SUBACUTE BACTERIAL ENDOCARDITIS TREATED WITH SULPHANILAMIDE RESULTING IN GRANULOCYTOPENIA AND DEATH*

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The recent therapeutic success of sulphanilamide in many coccal as well as other infections has led to its widespread and sometimes injudicious use. The number and variety of recorded untoward reactions is doubtless considerably less than their actual occurrance, and the possibility of a para-aminobenzenesulfonamide exerting a toxic effect on the bone marrow because of its contained benzene structure suggests the importance of a careful study of the blood during therapy. Although the amount of sulphanilamide and its rate of absorption into the blood can be accurately determined, there is little advance indication as to the individual susceptibility to a standard dose, particularly in severe general infections where the disease processes themselves may somewhat obscure the effects of the drug. Reduction in the number of circulating granulocytes has not infrequently been observed and four fatal cases have been recorded in the European² and five in the American literature to date^{2a}. None of these reports however demonstrate the morphological changes occuring in the bone marrow or other organs. That the granulocytopenia may be progressive in character following massive dosage and continue its course to a lethal outcome despite the rapid excretion³ of the drug is borne out by the following example.

P. E. F., a 39 year old white male accountant, was admitted to St. Luke's Hospital on March 17, 1938, complaining of easy fatigability, anorexia, and loss of ten pounds in weight, beginning a month before admission, and complicated by chills, sweating, and remittent fever, with temperature excursions to 105 during the latter two weeks. Prior to the onset of his present condition he

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had always been in good health. There was no history of previous rheumatic fever or significant illnesses. Physical examination showed a fairly well developed and nourished individual with a slightly enlarged heart and a loud, presystolic murmur localized over the apex. The spleen was not palpable; no petechiae were noted. The blood count on admission showed 80 per cent. hemoglobin, 5,160,000 red blood cells, 25,500 white blood cells, with 88 per cent. polynuclear leucocytes, 9 per cent. small lymphocytes, and 3 per cent. large lymphocytes.

A blood culture on the day of admission showed a growth of Streptococcus viridans. Because of the almost inevitable fatal progress associated with bacterial endocarditis of this type, massive treatment with sulphanilamide was instituted as a possible means of sterilizing the patient's heart lesion and circulating blood. No associated treatment was prescribed, other than a transfusion of 350 cc. of whole blood on the day of admission, and 500 cc. of the 26th and 29th days of hospitalization.

Therapy consisted of prontylin, 15 grains every three hours by mouth, totalling 120 grains in 24 hours, starting on March 21, 1938, the fifth day of hospitalization. On April 1, 1938, after a total of 1200 grains (80 grams) the dosage was increased to 160 grains daily, and on April 7, 1938, again raised to a total of 180 grains every 24 hours. On April 13, 1938, after having received a total of 4,240 grains (282.6 grams) of prontylin in 23 days, an abrupt fall in both the total number of leucocytes and the relative percentage of neutrophils was noted, and the drug withdrawn.

Table 1 shows the blood picture and reaction preceding, during, and following the withdrawal of prontylin therapy.

Throughout the hospitalization the patient presented the appearance of chronic sepsis without unusual mental or physical manifestations. Cyanosis or jaundice were never present. A bile index on April 18, 1938, the 32nd day of hospitalization, was 7. Urinalysis on three occasions showed a faint trace of albumin and on one occasion was associated with rare hyaline and granular casts. A fleeting pink macular rash, not fading on pressure, was noted on April 13, 1938, the 24th day following admission, but disappeared without sequelae the following day. Electrocardiograph tracings showed no abnormalities.

During the first week of hospitalization the temperature undulated gradually from 99 to 103, with similar variations during the second and third week from 100 to 102.6. More abrupt daily variations between 103 and 105 were noted during the fourth and fifth weeks with direct rise to 107.2 just preceding death.

An autopsy was performed six and a half hours after death.

The heart weighed 325 gm. Both cusps of the mitral valve were uniformly moderately thickened, particularly at their free borders. Toward the midportion of the posterior cusp, a soft pliable reddish vegetation 1 cm. wide was firmly adherent to the free valve edge. Small thrombi were present on the

auricular endocardium above the posterior mitral cusp. The chordae tendineae were thin and pliable. The valve appeared slightly insufficient, but no stenosis was evident. The remaining valves were intact, but no noteworthy changes were present in the myocardium or coronary vessels.

The lungs were moist and deep reddish grey in color. Throughout the left lower lobe were scattered raised round greyish firm granular areas about 1 cm. in diameter of pneumonic infiltration.

The liver weighed 1750 grams; its capsule was smooth and glistening. The parenchyma was pale yellowish brown and somewhat glazed in appearance. No distinct hemorrhagic or necrotic foci were grossly discernible. The gall bladder and extrahepatic ducts as well as the portal vein radicles were normal.

TABLE 1

BLOOD CULTURE	LARGE LYM- PHOCYTES	SMALL LYM- PHOCYTES	POLYNUCLEAR LEUCOCYTES	WBC	RBC	нвс	DATE
	per cent	per cent	per cent			per cent	
Positive strept. viridans	3	9	88	25,500	5,160,000	80	3/17
130 colonies strept. vi- ridans per plate	4	16	80	11,700	3,100,000	50	4/7
	1	10	89	11,200	3,800,000	53	4/9
		48	52	4,600	3,850,000	52	4/11
300 colonies of strept viridans per plate		96	4	800	3,100,000	45	4/13*
		98	2	700	3,330,000	60	4/15
		100		350	3,250,000	50	4/17
		100		200	3,400,000	51	4/18

^{*} Prontylin discontinued.

From 3/21 to 4/13 a total of 4,240 grains (282.6 grams) of prontolyn in 23 days. The red cells showed no morphological changes.

The bone marrow of the sternum and vertebral bodies was deep red in color, and firm in consistency. The marrow of the femur was fatty throughout.

Microscopic sections from the mitral vegetation show clumps of fibrin containing bacterial clumps covered only by a thin layer of hemorrhagic exudate. The myocardium is edematous; the capillaries engorged. Occasional minute areas of atrophy are present. There is some perivascular hyaline scarring, but no Aschoff bodies are present (fig. 1).

The pneumonic areas examined in the left lower lobe consist of fibrin and debris-filled alveoli enclosing clumps of bacteria and occasional desquamated septal cells. No neutrophilic or leucocytic elements of the circulating blood are present (fig. 2).



Fig. 1. Fibrin-enmeshed Clumps of Streptococcus viridans Covered with Hemorrhagic Exudate. \times 375

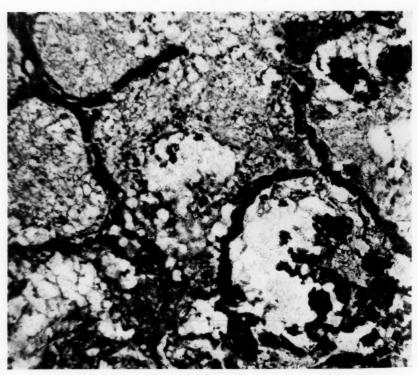


Fig. 2. Bacterial-filled Alveoli with Absence of Leucocytic Reaction. \times 180

The liver lobules are clearly outlined and have regularly arranged portal systems, some of which show slight lymphocytic infiltration. The central veins are dilated. The intermediate zones of the lobules contain scattered small areas of necrosis infiltrated with occasional small lymphocytes and plasma cells. The remaining liver cells show slight granular degeneration, but no other lesions.

Sections from numerous areas in both sternal and vertebral marrow present a uniform appearance. This is characterized by patchy irregular areas of aplasia alternating with small foci of myeloid hyperplasia. The aplastic zones

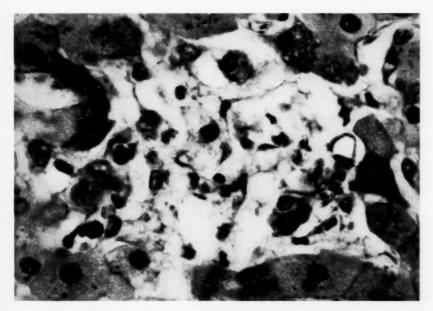


Fig. 3. Intermediate Zone of Liver Lobule with Area of Focal Necrosis \times 875

show large sinusoids filled with mature red cells, many of which are undergoing disintegrative changes. Megalo- and normoblasts about the sinusoids are moderate in number, but predominating the fields are small lymphocytes and occasional plasma cells (figs. 4 and 5). Adjacent clumps of myeloid hyperplasia border on the aplastic zones and about dilated blood vessels. They are composed chiefly of myeloblasts and scattered megalo- and normoblasts with a few lymphocytes and plasma cells (figs. 6 and 7). No mature polynuclear cells were observed in any of the fields nor are earlier stab forms or mature myelocytes to be noted. A few mitoses are present. Megakaryocytes are reduced in number. No thrombi are present in the vessels, nor are bacteria demon-

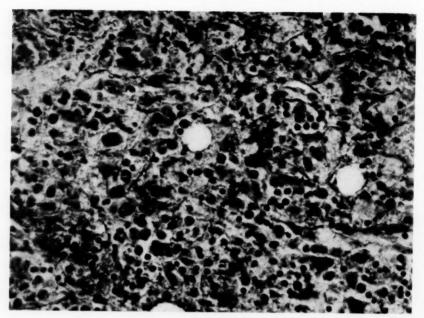


Fig. 4. Vertebral Bone Marrow: Aplastic Zone with Increase in Small Lymphocytes and Plasma Cells. \times 375

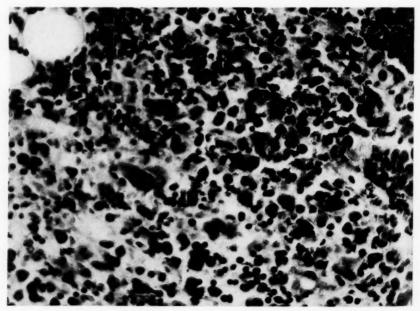


Fig. 5. Vertebral Bone Marrow: Area Showing Character of Cells in Lymphocytic Foci about Engorged Blood Vessels. \times 375

strable by special staining. The disintegrative changes in the red blood cells, as well as the occasional clumping noted in some of the vessels, appear the result of postmortem change.

The marrow from the fe:nur shows only fat cells.

This patient suffering from an acute disease in which blood studies constantly show a moderate leucocytosis was given massive doses of prontylin with no other therapy than an oc-

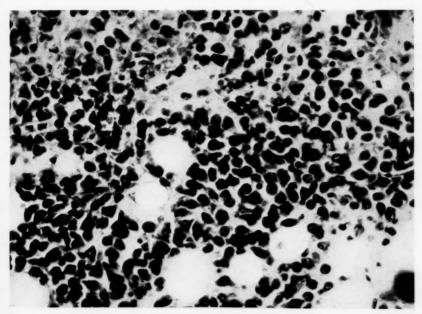


Fig. 6. Vertebral Bone Marrow: Foci of Myeloid Hyperplasia Bordering on Aplastic Zones. $\times\,375$

casional blood transfusion. The resulting changes in the bone marrow are presumably the result of the toxic action of the sulphanilamide. While hyperplastic, aplastic and reticulo-endothelial cell proliferation are usually described as clear-cut pathological entities occurring in the bone marrow in agranulocytosis, the case presented does not permit any such clear-cut pathological definition, but shows a combination of the hyperplastic and aplastic types with the latter changes predominating. The

increase in small lymphocytes to our viewpoint can scarcely be regarded as a hyperplasia of the reticulo-endothelial system in the ordinary sense⁴. Whether the foci of myeloid hyperplasia represent maturation arrests or inhibition of stimulation to the production of riper forms of myelopoiesis caused by the toxic action of the drug is difficult to state on grounds of pure morphology. Should these aplastic areas represent primary sites of damage with resultant hyperplasia of the remaining myelo-

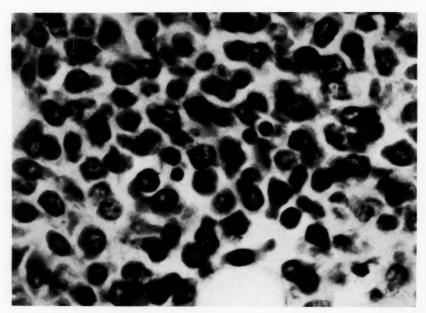


Fig. 7. Vertebral Bone Marrow: Same Area Showing Myeloid Hyperplasia. × 875

plastic or uninvolved tissue which in turn is destroyed before advancing to maturation, it would seem that the erythropoietic tissue would be affected to the same degree. Such areas of red cell destruction are not present, nor was the bile index clinically increased. Also should these changes be the result of embolic phenomena produced by the primary disease process, ample morphologic changes would be in evidence.

Other changes apparently the result of toxic action of the drug

are seen in the focal necrosis of the intermediate zone of the liver. These are bacteria-free on staining, and no thrombi are noticed in the adjacent vessels. Such changes are unaccompanied by any exudative reactions as one might expect with the total absence of circulating leucocytes. That they are due to the toxic action of the streptococcus viridans in the circulating blood is also unlikely, as is borne out by autopsy reports in this disease, unless the result of septic emboli. The pneumonic changes, on the other hand, judging from the clumps of bacteria enmeshed in fibrin, appear to represent an actual pneumonitis with necrosis but without cellular exudative phenomena due to lack of leucocytes.

SUMMARY

1. A case of Streptococcus viridans endocarditis was treated with large daily doses of sulphanilamide.

2. Following the ingestion of 4,240 grains (282.6 grams), administered over a period of 23 days, an abrupt fall in both the total number of leucocytes and relative percentage of neutrophilic polymorphonuclears occurred, becoming progressively more marked, and resulting in death.

3. Bone marrow studies showed alternating areas of aplasia and myeloid and lymphocytic hyperplasia with the former lesion predominating. Focal areas of necrosis were present in the intermediate zone of the liver. Pneumonic lesions in the lung were unaccompanied by any leucocyte response.

4. We believe the pathological changes followed the sulphanilamide therapy and were not caused by the original disease

process.

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EXPERIMENTAL TISSUE LESIONS WITH MIXTURES OF HUMAN FAT, FATTY ACIDS, SOAPS, AND CHOLESTEROL*

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Certain focal lesions in tissues and some systemic disorders are ascribed to deposits of lipids¹. When the lipid material can be demonstrated by suitable stains, the etiologic correlation with the lesions seems simple; but after the tissue reactions have reduced the lipids to minute or chemically modified particles. the noxious substances are not so readily identified in the lesions. Even when the presence of lipids can be demonstrated, the exact nature of the substances giving the stimulus for the tissue reactions is not always understood. The development of inflammatory reactions in subcutaneous and other fat tissues, which Makai designated as lipogranulomatosis, has been described in a number of reports. According to Abrikosoff, the earliest lesions are focal necroses of fat tissues and subsequent saponification. Later, the inflammatory response is a growth of fibroblastic tissue containing lymphocytic and leucocytic exudates. Older lesions contain fat droplets with marginal giant cells. In chronic lesions the granulation tissues become a fibrous scar with large vacuoles.

Neutral fats are the main constituents liberated from fatty areolar tissues. Opinions differ as to whether the neutral fats themselves, their split products, the fatty acids, or added substances such as cholesterol and phosphatides are the important elements in the stimulation of the tissue reactions. Wail observed cellular infiltrations about human fat injected subcu-

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taneously into the tissues of rabbits. Later, the tissue response included fibroblasts and giant cells. He noted a gradual increase of cholesterol and phosphatide compounds in the injected fat material. According to the view shared by Makai, von Quierke and Askanazy, the fat liberated by necrotic cells is split rapidly into fatty acids. The fatty acid content is the irritating factor which stimulates the lipophage reaction. Ansart and Got. however, expressed the view that the unsplit neutral fat provokes the inflammatory reaction. Sabin and her associates studied the tissue reactions produced by lipids extracted from tubercle bacilli, and correlated the chemical composition with the type of tissue response. The lipid material from tubercle bacilli was fractioned into three portions. The first, soluble in acetone, was a mixture of many fatty acids so irritating that it stimulated every type of connective tissue cell. The other fractions, the phosphatides and the unsaponifiable material, the waxes, stimulated the monocytes. With phosphatides, the monocytes developed into epithelioid cells which formed the typical tubercle; with the waxes, they fused into foreign body giant cells.

Emphasis has been placed on the action of some fatty acid content in explaining the effects of fats liberated in tissues but no study has been made to determine what effect soap compounds of various bases may have on inflammatory reactions. Neutralization of rancid olive oil or other oil mixtures by an aqueous solution containing an acid binding substance is well known commercially. Soaps thus formed and soluble in water are removed by washing. If the soap compound is much less soluble in water, conditions of equilibrium are established at the oilwater interphase that have not been studied thoroughly.

In experiments performed in a test tube, oleic acid was shaken with an aqueous solution of calcium hydroxide containing the indicator phenolsulphonthalein (phenol red). The color of the indicator first showed alkalinity in the aqueous solution, then shifted to acidity, demonstrating the entrance of H-ions from the oil phase into the aqueous. Presumably, an equivalent amount of base was transferred from the aqueous to the oil phase. The migration of cations into oleic acid may be observed with aqueous

solutions of ferric chloride. Oleic acid shaken with such a solution assumes rapidly the red-brown color of ferric compounds, and the original intensity of the color of the aqueous ferric salt solution decreases.

Years ago, Oscar Klotz embedded capsules containing fat and fatty acids in animal tissues. After several days, the fat material contained calcium soap and the calcium content was much greater than that of normal blood and lymph. Recently, Langmuir and Schaeffer found that monomolecular films of stearic acid floating on water containing calcium or barium salts were converted completely or in part into the corresponding soaps of the fatty acids and that the extent of the reaction depended upon the concentration of hydrogen ions in the aqueous solution. Paul J. Hartsuch² in our laboratory investigated the chemical reaction between oleic acid and aqueous solutions of calcium and especially of magnesium. The chemical reactions between sodium or potassium hydroxides and a fatty acid such as oleic acid are well known. In the presence of considerable excess of oleic acid, the sodium soap distributes itself between the aqueous and oil phase. With calcium hydroxide and an excess of oleic acid the relations are different. The calcium oleate formed is insoluble in water but is readily soluble in oleic acid. The calcium oleate accordingly, at equilibrium, is found in only the oil phase. Fluids in the animal tissues do not contain free bases such as sodium hydroxide or calcium hydroxide. The reaction, therefore, between oleic acid and dilute aqueous solutions of magnesium acid phosphate (MgHPO₄) was investigated.

His results demonstrated clearly that a considerable percentage of magnesium was lost from a solution of magnesium phosphate that contained 0.02 to 0.1 gram Mg per liter, and was converted into magnesium oleate which dissolved in the excess of oleic acid present. Simultaneously, H-ions were transferred from the oleic acid to the aqueous solution. The importance of the pH of the aqueous phase in these reactions was also apparent. Little reaction took place when the pH of the aqueous solution was 5.7 but as the pH increased a corresponding larger percentage of magnesium was transferred from the aqueous solution

to form magnesium oleate dissolved in the excess of oleic acid present. These reactions between oleic acid and alkaline aqueous solutions of magnesium explain the accumulation of magnesium, calcium and other bases in pathologic tissues rich in fats.

The observations mentioned developed simultaneously into a study of the tissue reactions produced in the lungs of rabbits by intravenous injections of human fat alone and of human fat containing oleic or stearic acid without further chemical treatment and after contact with aqueous solutions of calcium, strontium and barium hydroxide respectively.

Fatty acids of oil systems in contact with alkaline aqueous solutions exchange H-ions for basic ions dissolved in the aqueous The fatty acids in such oil systems are solvents for soaps insoluble in aqueous solutions. Human fat containing fatty acids abstracts basic ions from aqueous liquids in contact. soaps so formed are important in determining the subsequent tissue reactions. When an oil contains dissolved fatty acid, the exchange of hydrogen and basic ions at the oil-water interphase is an important link in the chemical reactions between the two immiscible systems. Human fat with a comparatively high content of oleic or stearic acid, produced in the lungs of rabbits a marked fibroblastic tissue reaction, and the stearic acid mixture, some foreign body giant cells. Human fat containing small concentrations of oleic and stearic acids neutralized with calcium hydroxide stimulated leucocytic exudates and a moderate fibroblastic tissue response. After neutralization with strontium or barium hydroxide, this mixture stimulated a marked reaction of fibroblastic tissue and epithelioid and giant cells.

Dr. Cornelius Hagerty³ extended the lung tissue experiments by injecting fine suspensions of mixtures of human fat, fatty acids and soaps into the renal arteries of rabbits and dogs in order to determine the effects on the kidneys. He found that the emulsions of human fat affected the kidney tissues in two respects. (1) The lipid material caused tissue reactions in the glomeruli, the nature of which depended upon the chemical composition of the material injected. (2) The obstruction of the glomerular tufts by the droplets, the reactive tissues or both

caused atrophy, necrosis, or fatty changes of the cells lining the tubules and a growth of interstitial connective tissue. Oleic acid, a strong irritant, caused swelling and necrosis of the endothelial cells and a marked growth of collagenous tissue that rapidly obliterated or replaced the usual glomerular structures. Liquid petrolatum, a weak irritant used as a control, after many days caused a hyperplasia of the endothelial cells with only a few collagenous fibers. The cellular response was local and limited to the direct proximity of the droplets. Human fat alone was a moderate irritant in the tissues of the glomerular tufts and produced a moderate proliferation of the endothelial cells associated with some fine and coarse fibers. Human fat containing oleic acid neutralized with calcium hydroxide was a strong irritant but not so effective as the oleic acid. Human fat containing stearic acid alone or with oleic acid and neutralized with calcium hydroxide produced reactions similar to but not so marked as those caused by human fat and oleic acid.

The response of the glomerular tissues to these substances was of two types, depending upon the nature of the substance injected. One was an endothelial proliferation; the other, a growth of collagenous connective tissue. Weak irritants caused hyperplasia of the endothelial cells of the glomerular tufts and a minimal production of collagen. Strong irritants caused endothelial swelling and marked formation of collagenous tissue. Mild or moderately strong irritants produced hyperplasia of endothelial cells and a moderate growth of collagenous tissues. The strength of the irritant seemed to determine the speed with which the lesions were produced.

The lesions thus produced were similar to those seen in diffuse glomerulonephritis in man. The rôle of fats as a possible etiologic agent in Bright's disease apparently has not been suggested. The results of the experiments indicate a possible etiologic relation.

The effects of mixtures of human fat, fatty acids, soaps and cholesterol were then tested. The cholesterol was extracted from human gall stones and purified by recrystallization. At 37.5°C. approximately 4 per cent of this cholesterol preparation

is dissolved in human fat. Intravenous injections into rabbits repeated frequently during two months did not influence appreciably the cholesterol content of the blood of these animals. There were only a few focal aggregates of exudates in the lungs. In one rabbit of three there were several small fibrous lesions with one or more foreign body giant cells containing acicular slits, an indication that some of the cholesterol material had crystallized from the fat solvent and had been encapsulated. Oleic acid or stearic acid in human fat markedly increase the solution of cholesterol. Human fat with an equal volume of oleic acid dissolves 13 per cent cholesterol at 37.5°C. Frequent intravenous injections of this mixture in rabbits from one and one-half to three months did not increase the cholesterol content of the blood and produced only slight focal changes in the lungs. The results with human fat containing 8.4 per cent stearic acid and 12 per cent cholesterol at 37.5°C, were similar. Mixtures of this kind, however, layered over calcium hydroxide at 37.5°C. so that the fatty acids were neutralized, then produced nodules of granulation tissue containing giant cells with acicular slits in the lungs of rabbits, indicating that cholesterol had crystallized from solution.

At least three factors, alone or combined, have a rôle in the causation of lesions with mixtures of human fat and the products of their hydrolysis. The first is the intensity of the acidity which develops during hydrolysis by the escape of acid ions from the oil phase into the tissue fluids. If sufficiently intense, this acidity causes necrosis of the tissues. The second factor is the nature of the soap compound formed in or about the oil phase during the chemical reaction between the fatty acid and the surrounding aqueous fluids of the tissues. Soaps slightly soluble or insoluble in water accumulate in the oil system or are precipitated in the tissue fluids where inflammatory reactions occur dependent in character upon the base entering into the soap compounds. The soaps least soluble in aqueous solutions apparently produce the maximum tissue reactions. The third element comprises the substances dissolved in the oil phase, such as cholesterol, which are insoluble in aqueous solutions. These substances in

the usual process of oxidation may be utilized completely. With varying conditions they are not, and remain as insoluble residues, crystalline or otherwise, when the solvent fats, notably those liquid at body temperature, are removed. An approximately saturated solution of cholesterol from human gall stones in human fat (roughly 4 per cent at 37.5°C.) is removed rapidly in the circulation of rabbits without appreciable lesions in the lungs and other viscera. Human fat slightly supersaturated with cholesterol at body temperature and injected intravenously stimulates in the lungs fibroblastic lesions with foreign body giant cells. Fatty acids markedly increase the solvent property of human fat for cholesterol. Such mixtures with a high content of cholesterol also are utilized rapidly in the circulation of rabbits without tissue reactions. These mixtures become semisolid by contact with aqueous solutions of calcium hydroxide. semisolid substances produced in rabbits fibroblastic lesions containing foreign body giant cells with acicular slits, the loci of cholesterol crystals.

In evaluating tissue lesions ascribed to depots of lipid-cholesterol mixtures, the factors leading to supersaturation of the solvent with cholesterol are important. The chemical disturbances leading to supersaturation with cholesterol in such systems may center not on the utilization of the cholesterol material but on the hydrolysis and oxidation of the solvent fat medium.

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PATHOLOGIC CHANGES PRODUCED BY CHLORINATED LARD*

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It was noted by Halvorson, Cade and Fullen^{1,2} that chlorinated packing house waste was toxic to animals. It was apparent that this toxic agent resided in the ether soluble fraction and therefore, in all probability, represented a chlorinated aliphatic compound. It is the purpose of this investigation to determine the toxicity of chlorinated lard, and the changes produced by it.

The toxicity of chlorinated alkyl radicals has long been known. Evarts Graham³ made a very complete investigation of poisoning with chloroform and other alkyl halides and found the wellknown changes which consisted of fatty degeneration and necrosis of the liver. He considered that these changes were brought about by the hydrolysis of these compounds in the liver with local production of HCl. K. B. Lehman⁴ studied chronic poisoning of a number of animals with alkyl halides. He found fatty degeneration, passive congestion of the liver with CHCl₃, CCl₄, and C2Cl4 but no distinctive pathologic change with trichlorethylene. H. C. MacMahon and S. Weiss⁵ reported a case of an alcoholic who drank an ounce of CCl4. There was extreme fatty degeneration of the liver and kidneys. The blood in the pulmonary artery showed enormously more fat than on the left side of the heart which they believed was derived from the liver and filtered out by the lungs. A. M. Lecquet found that regenerated liver tissue, after partial hepatectomy, was more resistant to CCl4 than normal liver tissue. R. M. Anderson7 found essentially the same results with CHCl₃. P. D. Lamson, B. H. Robbins and C. B. Ward have discussed the pharmacology and toxicology of tetrachlor-ethylene which has been used as an

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anti-helminthic in place of CCL and found that this produced no definite pathologic change in dogs. They found, however, some fatty change in livers of cats and puppies. These authors showed that this substance has much less toxicity than CCl. G. S. Barsaum and K. Saad a carried out a very thorough investigation of the relative toxicity of alkyl halides. In acute poisoning by oral administration to dogs they determined the M.L.D. in grams per kilo. This table is reproduced here together with the values of M.L.D. of the actual chlorine present in grams per kilo as computed by us from their results.

	M.L.D. OF COMPOUND	M.L.D. OF CHLORINE	
	grams. per kgm.	grams Cl per kgm.	
CH ₂ Cl ₂	3	2.5	
CHCl ₃	2.25	2.0	
CCl4	4	3.7	
C ₂ H ₄ Cl ₂	2.5	1.8 = Mean 1.8	
C ₂ H ₃ Cl ₃	0.75	0.6	
C ₂ H ₂ Cl ₄	0.70	0.6	
C2HCl5	1.75	1.5	
C2Cl6	No deaths		
C2H2Cl2	5.75	4.2	
C2HCl3	No deaths		
C2Cl4	No deaths		

It is seen that in the methane series, the relative toxicity is not proportional to the chlorine content, although, when the molecular equivalents are computed, CH₂Cl₂ is considerably less toxic than the two higher of the series. There is similarly no uniform relationship between the chlorine content and the toxicity of methane derivatives. C₂H₂Cl₂ is the least toxic. This variation may be due to variations in the absorption of these compounds; however, variations are also found in the intravenously administered substances. The toxicity of aromatic chlorine compounds has been mentioned by Hamilton¹⁰ who states that association of chlorine with aromatic compounds does not increase its toxicity. In fact, chlor-benzene is less toxic than benzene. This, however, is in contrast to the work reported by G. R. Cameron and J. C. Thomas¹¹ who found that the relative toxicity of benzene and its derivatives by subcutaneous administration was as follows:

 ${\bf Benzene} \, < \, {\bf Monochlorbenzene} \, < \, {\bf P\text{-}dichlorbenzene} \, < \, {\bf O\text{-}}$ ${\bf dichlorbenzene}$

Benzene > Trichlorbenzene > Tetrachlorbenzene > Hexachlorbenzene

It is thus seen that the toxicity of chlorbenzene increased up to two chlorine atoms and after that decreased. Furthermore, there was a difference in toxicity between para and the ortho positions. Thus it is seen, as in the case of the alkyl compound, that the toxicity is not uniformly dependent on the chlorine content.

The causes of fatty degeneration are not clearly understood. Graham³ believed that it was due to a local production of HCl in the liver. Fisher¹² believed that fatty degeneration is caused by impaired oxidation in the liver, since substances which produced fatty degeneration are known to have a general protoplasmic effect. As a result of this lowered oxidation in the liver, there is a local acidosis produced which changes the colloidal character of the cells so that fat formerly completely emulsified is liberated in droplets. There is no actual increase in the fat present. M. E. Hanke and P. B. Donovan¹³ determined the total chloride and organic chloride for the liver to be 0.3 and 0.35 per cent, the organic chloride from 0.15 to 0.16 per cent.

EXPERIMENTAL

For these experiments, non-hydrogenated commercial lard was used. The lard was melted in a large flask closed with a stopper containing a tube through which chlorine gas was admitted. The lard was kept at 80°C. in a water bath and shaken violently periodically. The chlorination required about two hours for apparent conclusion. At the end of this time there was no further observable absorption of chlorine. It was felt that this represented complete saturation of the fat but evidently under these circumstances this was not true, since it was found upon analysis that the chlorine content of the fat was far below the theoretical saturation point as computed from the iodine number of lard. The chlorinated lard was then washed repeatedly in a separatory funnel with distilled water until the washings gave no test for chlorine, then separated

from the water in a separatory funnel, and then placed in a large evaporating dish and heated for one-half hour at 100°C. A portion of it was then analyzed for chlorine. The mechanism of saturation of chlorine is similar to that of iodine. It associates itself with the fatty acids of the fat for the most part by combining with unsaturated bonds, but to some extent by replacement of hydrogen attached to saturated carbon atoms.

The Carius method of analysis for organic chlorides was used¹⁴. A large heavy pyrex tube was used which was heated at 160°C, for 24 hours. Although this temperature was lower than recommended, yet over this period of time it resulted in complete decomposition of the fat. Considerable difficulty was encountered when the tubes were opened due to the violent evolution of dissolved gases. It was very difficult to control this, but by the use of low temperatures it was found that this difficulty could be entirely avoided. The tubes were set in a vertical position and surrounded by CO2 snow. In a very short time the contents of the tube was frozen in a semi-solid form and the brown N₂O₄ which had filled the upper part of the tube had completely disappeared due to condensation. When the tube was opened, there was only a slight amount of gas released which, however, was sufficient to cause the contents of the tube to freeze solid as a result of the decompression. The tubes were then allowed to stand in CO₂ snow which gradually evaporated so that the liquid melted progressively from above downward. This entirely prevented any tendency to bubble over, since gas was not prematurely released in the lower part of the tube by rapid and unequal warming. The analysis of the fats used are listed in table 1.

The series of rats were fed the chlorinated fat no. 1 spread on bread together with their regular diet. Within two days, one rat who became sick at the very first, died. It was then found that the other rats were not eating the chlorinated fat. Some of the fat was then melted and stirred up with corn meal and dry oats, and this was given to the rats without any other food so they were obliged to eat this exclusively. The time of the death of the rats in days after the feeding was started is shown in table 2.

The symptoms of these rats were essentially similar. The animals soon became listless and weak, their appetite was diminished and their coats became dirty and ill-kept. It was found that their desire for normal food was so great that there was considerable mutilation of the carcasses of the rats dying in the group. During the last day of life, the rats were semi-comatose and could not be caused to move about by disturbing them. At this time their eyes appeared much lighter pink than normal. The gross autopsy findings on the rats were extremely similar. It is therefore not considered worth while to give a detailed summary of the results since with one exception they were identical. In general, the results may be generalized as follows:

The liver was found to be soft, friable and congested. Cut section showed a well-defined architecture with a yellow-grayish appearance. The kidneys

were soft, swollen, yellowish in appearance and quite congested. The heart showed terminal right sided dilatation. Cut section showed edematous light musculature. The lungs in some cases showed patchy atelectasis. The spleen and abdominal viscera showed acute passive congestion. The brain showed no abnormalities.

Gross pathologic diagnosis: (1) fatty degeneration of the liver; (2) cloudy swelling and fatty degeneration of the kidneys; (3) fatty degeneration of the heart; (4) terminal acute passive congestion of the abdominal viscera; (5) atelectasis of the lungs. Of this series, rat 2 showed very peculiar changes

TABLE 1

	PER CENT Cl	PER CENT SATURATION	Sp. G. $\frac{30^{\circ}}{25^{\circ}\text{H}_{2}\text{C}}$
		per cent	
Chlorinated fat			
1	4.6	17.8	0.93
	4.7		
2	7.8	30.1	
	7.8		
Untreated lard	0.02		
	0.02		

TABLE 2

	AGE	NO. OF DAYS
1	Adult	2
2	Young	2
3	Young	3
4	Young	4
5	Young Young	6

since these degenerative changes had proceeded to necrosis. This rat died at night and was autopsied not more than twelve hours after death. The abdomen was grayish in color and the viscera were matted together giving it the appearance of moderate post-mortem change. The free border of the liver was grayish in color, semi-liquid in consistency and showed no architectural pattern. The kidneys were very soft, almost semi-liquid, and yellowish in color. There were small patches of necrosis on the peritoneal covering of the liver, on the pleura and on the kidney capsule. The heart was congested and grayish-purple in color.

The second series of rats was started using chlorinated fat no. 1. A definite

amount of the melted fat was introduced into the upper part of the esophagus by means of a small catheter. The fat was administered daily with the exception of Sunday. The young rats used for this experiment were all the same age and nearly the same weight (which averaged 65 grams). The results in this series are shown in table 3.

This series of rats showed essentially the same gross pathological changes as the first, however, even the acutely poisoned ones showed no instances of frank necrosis. Rats F and H, killed eleven and sixty days respectively after the discontinuance of the feeding, showed no gross changes.

The microscopic changes in both series of rats were very similar, although differing in amount in various individuals. Ordinary hematoxylin and eosin stains were made of the liver, heart and lungs, and fat stains were also made of the other organs including the brain in about half the animals. These showed

TABLE 3

RAT	NO, OF DAYS	DOSE P	ER DAY	TOTAL DOSE	
BAI	NO. OF DATE	Fat	Cl	Fat	Cl
		cc.	cgs.	cc.	cgs.
A	2	2	8.6	2	8.6
В	$2\frac{1}{2}$	2	8.6	2	8.6
C	8	1	4.3	7	30.1
D	10	1	4.3	7	30.1
E	3	1/2	2.2	1.5	6.5
F	Killed 11 days after discontinuing fat	1/2	2.2	1.5	6.5
G	14	14	1.1	3.0	12.9
H	Killed 60 days after discontinuing fat	1/4	1.1	3.0	12.9

no essential change except for some congestion of the spleen. The microscopic findings are outlined below:

The liver showed marked congestion of the central veins and the sinusoids. In most of the sections the fatty degeneration was extremely uniform, the globules were so fine and so evenly distributed that even under high power, the liver cells appeared almost uniformly pink. In many of the sections the degeneration was uniform throughout the lobule, in others there appeared to be intermediate or central distribution and in still other sections the fat globules were larger and relatively much less frequent than those described. The kidney showed no glomerular change other than congestion but there was a very marked finely granular fatty degeneration of the proximal convoluted tubules. In most of the sections, the fat stained clearly and as well defined small globules. In others, the globules were larger, less uniform in size, and fewer, so that in the section the tubules appeared only a light pink in color. The heart showed, in many cases, an extremely diffuse fatty degeneration, the fat globules being so

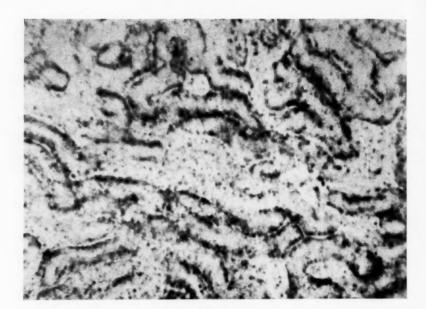


Fig. 1. Rat 1. Scharlach R. Stain. This shows fatty degeneration of the proximal convoluted tubules. The degeneration is quite extensive and marked. The fat droplets are small and uniform in size.

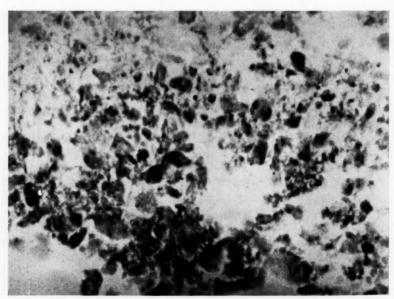


Fig. 2. Rat 2. H. & E. Stain. This shows a region of liver necrosis. Only irregular poorly defined cells are present whose arrangement shows little resemblance to liver architecture.



Fig. 3. Rat 4. Scharlach R. Stain. Fatty degeneration of the heart. Here the fat droplets are somewhat variable in size and density of distribution. The fatty change here is quite marked although not as uniform in distribution and size of fat droplets as in some of the heart sections.

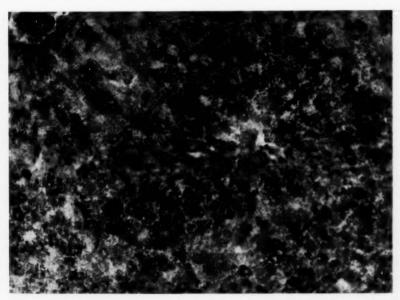


Fig. 4. Rat 5. Scharlach R. Stain. Fatty degeneration of the liver. The fat is deposited in the central and intermediate zones of the lobule. The fat droplets are of variable size. In many liver sections the droplets were smaller and uniformly distributed throughout the lobule.

fine that almost uniform appearance was seen under high power. In other sections, the degree of fatty degeneration in the heart was variable even in adjacent regions, and the globules themselves were larger. There was some edema of the interstitial substance.

The microscopic changes in rat 2 merit separate description. A section taken from the region of the necrosis of the liver showed such extensive damage that no cellular detail could be made out. There were, however, discrete collections of amorphous material containing some fat which by their general radial distribution suggested the structure of liver. The kidney showed almost as much destruction of its architecture as the liver. In many regions no tubules were definitely distinguishable. The section of heart showed very extensive fatty change and necrosis with very little remaining architecture. The changes in all these sections appeared to be entirely degenerative for there was little or

TABLE 4

	RAT	WEIGHT	WEIGHT OF LIVER	PER CENT OF LIVER WEIGHT	OF FAT IN LIVER	OF CHL. IN FAT
				Per cent	Per cent	Per cent
Chlorinated lard	i	159	14.2	8.9	4.9	0.35
Chlorinated lard	ii	195	15.8	8.1	6.3	0.25
Average	,			8.5	5.6	0.30
T1	iii	75	5.0	6.7	7.4	0.30
Lard	iv	165	16.5	9.8	6.2	0.27
Average				8.3	6.8	0.28

no evidence of inflammatory cell reaction. We, therefore, found that microscopic observations upheld the gross summary, namely, that the characteristic lesions produced by chlorinate fats were extensive and usually uniform fatty degeneration of the liver, kidney and heart. Rat F showed very slight remains of the fatty degeneration in liver, kidney and heart. Rat H showed no evidence of fatty change in these organs, although there appeared to be some slight swelling and some irregularity of tubular epithelium.

In an effort to determine whether or not there was actual deposition of the chlorinated fat in the liver, the following feeding experiment was arranged: A series of four young rats were used. Two of these were given ½ cc. of lard per day and two were given ½ cc. of chlorinated lard no. 2 for ten days. They were then killed, the livers removed and weighed. The livers were then macerated and extracted for five days with ten times their weight of alcohol-ether mixture. After this, the material was filtered and the residue extracted three times for a period of about an hour with fresh hot ether-alcohol mixture. The

filtrate was evaporated to dryness on a water bath and then weighed. It was then dissolved in ether and transferred to a large pyrex tube. The ether was then evaporated off and the chlorine determination made on the residue. The results are shown in table 4.

CONCLUSIONS

It is seen that the degeneration produced by chlorinated lard is essentially the same as that produced by chlor-alkyl compounds of low molecular weight described by previous investigators. Since rat A. and B. died almost immediately from a 2 cc. dose of fat, while rats receiving half that amount survived for a considerable time, this much chlorinated lard corresponds closely to the M.L.D. From this it was computed that for a young rat, the M.L.D. of chlorinated lard no. 1 is 28.6 grams. This was seen to be enormously larger than the values for the alkyl halides from the saturated series as reported by G. S. Barsaum and K. Saad. However, this was due to the low chlorine content of the chlorinated lard, for if the toxicity of the chlorine of lard is computed, it is found to be 1.3 grams per kilo which is in satisfactory agreement with the mean of 1.8 computed from the results of Barsaum and Saad. The analytical results on the liver showed that there was no significant variation in the percentage weight of the liver or in its fat content in normal and poisoned animals. This is in accordance with the general idea of Fisher¹² who pointed out that in fatty degeneration, the actual fat content of the liver is not increased. There was also no significant variation of the chlorine content of the fat of the liver. This indicated that there was no preferential deposition of chlorinated fat in the liver. This, combined with the known degenerative changes in the kidney and heart, uphold the idea of Fisher, that these changes are produced through a generalized effect on the oxidation process of cells rather than through a local hydrolytic process as suggested by Graham³. The results for the chlorine determinations on the liver organic fraction were considerably higher than that given by Hanke and Donovan¹³. Their technique of separation of the organic fraction was not described in the article. method used by us may very well have carried along some small amount of inorganic chlorine, not only from the tissue, but also

from the blood of the congested livers. Therefore, these results probably lie more nearly toward the correct values for total liver chlorine rather than that entirely associated with lipoids. At any event, the lack of significant variation in these figures are definitely against the storage of chlor-lipoids in the liver.

SUMMARY

- 1. The pathological changes produced by chlorinated aliphatic lipoids have been studied.
- 2. The quantitative relationship of the liver weight, fat content, and chlorine content has been determined.
- 3. The changes produced by the chlorinated fat was found to be essentially that produced by alkylchlorides of lower molecular weight.

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INFECTIOUS MONONUCLEOSIS*

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Infectious mononucleosis may be described as a disease characterized by fever (although this is not always present) lasting ten days to several weeks, lymph node enlargement, throat infection, headache, weakness and general malaise.

The spleen is probably enlarged in half the cases and in those cases not characterized by throat infection there may be a predominance of abdominal symptoms. It is also possible to have the combination of a throat infection and predominant cerebral symptoms and as intimated above, there may be an afebrile type.

Although "infectious mononucleosis" was the term applied to this disease by Sprunt and Evans¹ in 1920, there is still considerable argument as to its cause and amenability to tissue diagnosis. The question of differentiation from other diseases has been pretty well settled through the use of various methods which demonstrate the presence of heterophile antibodies in the blood and the question of treatment can be summed up in one sentence: Do nothing and they will all get well.

Forssman began the work in 1911 which culminated in a diagnostic test. At that time infectious mononucleosis, lymphatic angina, monocytic angina, and most of the other related diseases were grouped under the broad term "glandular fever."

As late as 1932 Selander² made the statement that the differential diagnosis between lymphatic leukemia and infectious mononucleosis rested on the favorable outcome of the latter, although in that same year Paul and Bunnell³ described the presence of heterophile antibodies in infectious mononucleosis.

At this time Hobson⁴ was attempting to differentiate excised

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lymph glands in this disease from other lympho-glandular pathology but found nothing in his tissue studies which would help in this differentiation, though he noted the increase in the neutrophiles in the blood stream at the onset. He also mentioned the presence of an enlarged lymph node in the posterior neck near the base of the skull.

In 1933 Rosenthal and Wenkebach⁵ reported twenty-eight cases, using the heterophile reaction to differentiate monocytic angina and glandular fever from infectious mononucleosis. This they were unable to do, probably because these diseases are very closely related, if not all varying types of the same disease. Since that time the technique of the test has been refined to such an extent that it seems unnecessary to resort to biopsy for diagnosis although Downey and Stasney⁶ claim that such a procedure is not difficult because of the relatively slight immaturity of the lymphocytes and the proliferation of the reticular cells in infectious mononucleosis.

In 1936 McKinley⁷, who reported fifty cases, quoted Kracke and Garver as stating that morphologically acute lymphatic leukemia and infectious mononucleosis cannot be differentiated with certainty and stressed the occasional presence of abdominal symptoms and the necessity for frequent blood counts to differentiate abdominal disease. He found throat involvement in 78 per cent of his cases.

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Certainly every laboratory should be able to perform one of the tests for the presence of heterophile antibodies in the blood stream because this disease can so easily be confused with: influenza, cervical adenitis, tonsilitis, lymphatic leukemia, Hodgkin's disease, syphilis, meningitis, tuberculous adenitis and meningitis, undulant fever, encephalitis.

Wulff's points to the necessity for ruling out diphtheria and stresses the differential count as being important in differentiating these two diseases. This is probably impractical because several days may elapse after the onset before the lymphocytes predominate.

In 1934 Lehndorff⁹ reported a small series of cases of "glandular fever" using the Paul and Bunnell technique which consisted of

making dilutions of 1:2 to 1:1024 of inactivated serum, mixing equal parts of these dilutions with 2 per cent washed sheep cells in saline, incubating in a water bath for one hour at 38°C. and refrigerating overnight before examining for agglutination.

In 1935 Davidsohn¹⁰ described a modified procedure in which the mixture was placed in 75 x 12 mm. test tubes and only refrigerated for 1 hour, thus permitted the reading of the test in two hours.

It was from Davidsohn that Paul and Bunnell got the idea which led to their original thirteen hour technique. He had previously been using the test in serum sickness and had found it positive in a high percentage of patients who had been given horse serum at any time. He has since been able to differentiate the antibodies developed from horse serum, which are true Forssman antibodies, from those of infectious mononucleosis by treating the serum with suspensions of guinea pig kidney. This treatment will remove Forssman antibodies but not affect the agglutination power of the serum of a patient suffering infectious mononucleosis. This finding may prove to be very valuable in cases of infectious mononucleosis which have received horse serum.

In our cases the technique described by R. Strauss¹¹ was used. This technique, although a modification of that of Paul and Bunnell, is considerably faster than even the two hour modification of Davidsohn. It follows:

- 1. Heat sera for one-half hour in water bath at 56°C.
- 2. Make dilutions from 1:2 and 1:1024 or more.
- 3. Place 1 cc. of diluted serum in test tube.
- 4. Add 1 cc. of 2 per cent suspension of washed sheep cells.
- 5. Centrifuge for five minutes at about two thousand revolutions per minute.
- 6. Shake and read.

The tubes are shaken by starting with the control tube, noting the vigor necessary to shake up the cells, then going to the tube with the highest dilution and working down. The lower dilutions show a single large red clump which is designated as four plus agglutination. Smaller clumps are designated three plus, two plus and one plus.

As Van Ravenswaay¹² points out, there are two possible explanations for the presence of these antibodies: (1) That they are

built by virus antigen action. (2) That they represent increased normally present agglutinins.

Regardless of the reason for their presence in the blood stream in this disease, sufficient work has now been done to prove

practically every case of infectious mononucleosis.

The height of the agglutination titer seems to depend on the stage of the disease, but for the first two or three weeks it is constantly increasing according to Butt and Foord¹⁸. Bailey and Raffel¹⁴ claim that ox cells can be used as well as sheep cells and agree with Davidsohn that the antibodies are not Forssman in type.

Wiseman, Doan and Erf¹⁵ call attention to the additional information to be gleaned from the red, white and differential blood count, and state that after the initial rise in leukocytes there is a steady decrease in the circulating granulocytes accompanying the rise in lymphocytes and that, as the lymphocytes increase, the red cells fall.

We have recently seen four cases at Huron Road Hospital and one in the office which, because of the possibility of mistake in diagnosis from the symptoms presented, caused us to make the above review of the literature.

The first case, A. P., presented the most difficult problem because his symptoms were predominantly cerebral. We have found only one other case of this type in the literature, that reported by Epstein and Dameshek¹⁶. We report this case in detail and give a short summary of the other two.

Case 1. A. P., a male, aged 29, was admitted to the hospital August 22, 1937 with chief complaints of headache, stiff neck, sore throat, weakness and general aching. Ten days previously he had noticed weakness and sore throat, which complaints had increased during the three days following onset when the patient was put to bed where he remained until the time of admission.

His symptoms increased in severity and he became drowsy and presented the appearance of having bilateral ptosis, but could open his eyes wide if requested.

On admission to the hospital photophobia was marked and the neck was moderately rigid. He complained of constant headache and the temperature for the previous seven days had ranged from 100° to 103°F. There was no history of cough or respiratory distress but there was marked swelling of the

lymph glands of the neck, especially the posterior cervical chains, and one nodule in the posterior neck near the occiput was especially large.

He answered all questions with effort and he did not articulate well. Ears and nose were normal. The eyes showed a horizontal nystagmus but the reflexes were normal. The mucous membrane of the throat was slightly injected and a provisional diagnosis of lethargic encephalitis was made.

The blood count at this time was red cells 4,000,000, white count 5,500, leukocytes 68 per cent, with 26 per cent juveniles, lymphocytes 30 per cent and large mononuclears 1 per cent. Spinal fluid at this time showed sugar 62 mgm. per 100 cc., cell count 0, colloidal gold 0000000000. The Kline test, diagnostic and exclusion, was negative. The urine showed a faint trace of albumin with rare pus cells.

Two days later the consultant neurologic diagnosis was "meningismus, as a result of toxic absorption produced by fever which is as yet of unknown origin but we suspect lymph glands." It might be added that by this time the inguinal and axillary nodes were prominent and sore.

The blood culture after 36 hours incubation was negative and two days after admission, August 24, 1937, the blood count showed: white cells 7,300, leukocytes 61 per cent and lymphocytes 37 per cent.

On August 25, 1937, he was obviously improved and because of this a second spinal tap was not done. The urine showed the same findings as the previous examination and the temperature began to go down.

On August 28, 1937, six days after admission to the hospital, the white count was 13,100, leukocytes 34 per cent, lymphocytes 62 per cent and large mononuclears 4 per cent. Up to this time the temperature had ranged between 103.6 and 99.4, the peak being reached each day at 8:00 p.m.

The tests for undulent fever, etc. were all negative. Because of the lymphocytosis, weakness and generalized adenopathy, an agglutination test for heterophile antibodies by the Strauss method was done and proved to be positive in a dilution of 1:512.

He was discharged from the hospital on August 29, 1937, was confined to his bed at home until September 10, 1937, and was able to come to the office on September 16, 1937, at which time he complained only of weakness. The glandular enlargement had subsided and his blood count was normal.

The treatment was purely symptomatic, consisting of hot packs to the neck, 50 per cent glucose intravenously, analyssics and sedatives.

Case 2. K. S., a male, aged 25, was admitted to the hospital on May 25, 1937, with a provisional diagnosis of acute lymphatic leukemia. He had never been sick, except for childhood diseases, until one week before when he noticed headache, afternoon rise in temperature, backache and sore eye balls. He developed some coughing, epigastric distress.

At the time of admission glands could be palpated in both the cervical and inguinal regions. The patient was anemic in appearance, thin, white and a

mouth-breather. The heart and lungs and abdomen were negative. Urinalysis was negative as was the chest x-ray. He complained of considerable weakness and the temperature was 104°.

The blood count at this time showed the hemoglobin to be 83 per cent, red count 4,500,000, white count 5,900, leukocytes 32 per cent, lymphocytes 62 per cent and large mononuclears 6 per cent.

During his stay in the hospital the temperature ranged between 104° and normal with no regular daily remissions, and fell to normal on the eighteenth day by lysis. Pulse was low in proportion to the temperature at all times.

Blood culture was negative but the white count increased in four days to 20,000 with 86 per cent lymphocytes and by June 8, 1937, the white count was 10,950 with 76 per cent lymphocytes.

The agglutination test for heterophile antibodies was positive in a dilution of 1:1024 by the Strauss method.

He was discharged June 14, 1937 with weakness his only important complaint.

Case 3. B. B., a female, aged 21, was seen October 19, 1937, with a chief complaint of sore throat of two weeks' duration. One day later the mucous membrane was so swollen as to permit some difficulty in respiration and there were large follicles on the tonsils. The neck showed anterior and posterior cervical adenopathy.

A smear and culture were negative for diphtheria. Four days after the onset a blood count showed the white cell count to be $16,\!000$, leukocytes 10

per cent, lymphocytes 80 per cent and mononuclears 10 per cent.

Five days after the onset an agglutination test for heterophile antibodies by the Strauss method was positive in a dilution of 1:256 and partially positive in a dilution of 1:512. Uninallysis was negative. The temperature was never higher than 99.4° and the pulse stayed below 100.

Eight days after the onset the patient was able to leave her home and shortly after that returned to school. She had no sequelae in the form of weakness or gland enlargement beyond two weeks from the onset.

Case 4. B. B., a male, aged 28, came to the office on October 18, 1937, with a history of feeling of warmth, lack of appetite, lassitude over a period of two weeks. He also complained of a mildly sore throat and post-nasal drip. His past history was non-contributory.

Physical examination showed a slight fever, 994, and enlargement of the posterior and anterior cervical lymph glands, mildly injected throat and a

post-nasal drip was evident.

Because of previous interest in infectious mononucleosis and a negative throat smear, a blood count was immediately taken which showed 70 per cent lymphocytes. On the strength of this a heterophile antibody test was made and was found to be positive in a dilution of 1:512. The differential count showed neutrophiles 27 per cent and mononuclears 3 per cent.

On October 25, 1937, one week after diagnosis was made, the temperature became normal and on October 29, 1937, all symptoms had ameliorated except the weakness and anorexia. At this time the hemoglobin was 80 per cent, the temperature was normal, throat was normal but there was still some glandular enlargement. By November 15, 1937, all symptoms had completely disappeared.

SUMMARY

Four cases of infectious mononucleosis are reported.

Each case simulated a different disease, one predominating in cerebral symptoms, one simulating acute lymphatic leukemia, one with all appearances of follicular tonsilitis and one with weakness the outstanding symptom.

All cases gave us positive agglutinations to sheep cells, the socalled heterophile antibodies by the Strauss method, completed within a few minutes after the blood was taken.

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THE WASSERMANN REACTION IN INFECTIOUS MONONUCLEOSIS*

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If one is to judge from the literature, infectious mononucleosis appears to be much more prevalent than in previous years. At the Michael Reese Hospital in Chicago, there were diagnosed three cases in 1934, four cases in 1935, seven cases in 1936, eleven cases in 1937, and five cases during the first 3 months of 1938.

Whether this increase in cases, here and elsewhere, actually is as great as it appears to be, or whether it is partly due to more exact diagnostic procedures is an open question.

In Paul and Bunnell's discovery in 1932 of the presence of antibodies against sheep red blood cells in the serum of patients suffering from the disease we have a diagnostic criterion of great accuracy. It is generally agreed that Paul and Bunnell's heterophilic antibody test is a specific test for infectious mononucleosis provided that serum sickness is ruled out².

In all the thirty cases of infectious mononucleosis reported at the Michael Reese Hospital during the last four years, the test was positive.

There seems to be no doubt that many cases formerly diagnosed as influenza, grippe, cervical lymphadenitis, aleukemic myelosis, and lymphadenosis are recognized now as infectious mononucleosis by means of this specific serologic test.

It is not intended to enter here into a review of the literature on this subject, which may be found in recent publications³, but to refer briefly to some communications pertinent to this study, and to report a case of infectious mononucleosis not only

^{*} Received for publication August 1st, 1938.

because of its serological aspects, but also because of its differential diagnostic difficulties. It is believed that this study may be helpful in recognizing and evaluating similar clinical manifestations of infectious mononucleosis.

REPORT OF CASE

Mrs. L. K., aged 32 years, was admitted to Michael Reese Hospital 3-20-38 with a history of being ill for the past week with fever up to 101, sore throat, backache, night sweats, lack of appetite and strength, as well as extreme fatique.

The past history was insignificant except that the patient had suffered a fracture of the humerus in a recent automobile accident for which she still

was wearing a splint.

Significant physical findings were those of temperature (1022), a mild sore throat with redness and follicular swelling, considerably enlarged painless anterior and posterior cervical nodes, and slightly enlarged submaxillary, axillary and inguinal lymph nodes. Heart and lungs were within normal limits. There were no pathological auscultatory findings except a soft systolic murmur over all the ostia of the heart. The spleen was moderately enlarged, palpable, one finger breadth below the costal margin, soft and not painful.

Significant laboratory findings were a leukocyte count of 9,300, differential count of polynuclear leukocytes-41, basophilic leukocytes-2, lymphocytes and large mononuclear leukocytes-57. The heterophilic antibody test was positive in a dilution of 1:32. There was a four plus Wassermann and a four plus Kahn test. Blood chemistry, blood culture and agglutination tests for

typhoid, dysentery and bacillus of Bang were negative.

During the next week the patient's condition remained stationary, the temperature not exceeding 1015, the Wassermann and Kahn reactions remained four plus positive on two subsequent examinations. The titre of the heterophilic antibodies rose to 1:64. At the end of the week the patient developed a generalized maculo-papular eruption with a rise in temperature to 103, the eruption involving particularly the chest, abdomen, back and extensor surfaces of the extremities. Another Wassermann test was four plus positive. The white count was 12,500 with differential count similar to the above. With the outbreak of the rash, the patient seemed to feel better.

The eruption gradually disappeared during the following week with a simultaneous decrease in temperature. The patient improved rapidly and regained strength and appetite. Serological examination shortly before the patient's discharge showed a negative Kahn test, a negative Wassermann test with cholesterolized antigen and a four plus Wassermann with lipoid antigen. The patient was discharged March 12th clinically recovered. A Wassermann and a Kahn test performed one week after discharge were completely negative throughout as well as a test taken 5 weeks thereafter.

COMMENT

From a clinical as well as from a serological viewpoint the patient offered unusual features. Clinically, the case presented a striking similarity to the pre-eruptive stage of primary syphilis. The presence of cervical, axillary and inguinal adenopathy, followed by a generalized maculo-papular eruption associated with four plus Wassermann and Kahn tests seemed almost conclusive evidence of a syphilitic infection. The prevalence of cervical adenopathy, a slightly enlarged spleen, and a low grade fever did not seem to contradict this assumption. However, the characteristic mononucleosis as well as the positive heterophilic antibody test left doubt as to whether we were dealing with infectious mononucleosis in a patient suffering from syphilis, or whether the positive Wassermann reaction was due to the presence of infectious mononucleosis.

The subsequent course, namely, the disappearance of the positive Wassermann reaction, made it clear that the patient was suffering from infectious mononucleosis accompanied by a positive Wassermann reaction.

The pitfalls the case presented are clear; had we failed to conduct the heterophilic antibody test, and had we failed to consider the characteristic blood findings, the patient doubtless would have been diagnosed as a case of pre-eruptive syphilis and thus would have been treated anti-luetically. The disappearance of the positive Wassermann reaction would then have been attributed to the result of the antiluetic treatment which raises the question whether cases of infectious mononucleosis clinically and serologically resembling pre-eruptive syphilis, may have been so diagnosed and treated erroneously in the past. If so, such cases seem extremely rare for none of the thirty cases in the series of infectious mononucleosis mentioned above, had a four plus Wassermann and Kahn reaction, and only two had a temporary one plus Wassermann reaction. None of the cases of this series presented any clinical resemblance to pre-eruptive syphilis.

What is the mechanism of the Wassermann reaction in infectious mononucleosis?

In a recent publication Hatz⁴ expresses the opinion that the presence of Forrsman antibodies in the blood of patients with infectious mononucleosis could be the cause of a positive Wassermann reaction, provided that the antigen used for the Wassermann reaction was prepared from the tissue of an animal in the Forrsman group, such as guinea pig or horse. This assumption cannot be accepted for this case since our Wassermann antigens were beef heart extracts. In addition, it seems doubtful as to whether the sheep cells antibodies in infectious mononucleosis are identical with the Forrsman antibodies. Results⁵ of inhibitory and adsorption experiments agree in showing that sheep cell antibodies in infectious mononucleosis are not Forrsman antibodies for they reacted differently in their immunologic behavior.

What, then, may be the cause of the positive Wassermann reaction in infectious mononucleosis? No satisfactory explanation can be given at present for this phenomenon. In analogy with similar serological conditions the author believes that infectious mononucleosis creates a disturbance in the protein fractions of the serum, which renders the serum "labil" and may be responsible for a non-specific protein reaction such as is seen in pneumonia, malaria, and pregnancy. While this explanation of necessity is of a hypothetical nature, it may be well to include infectious mononucleosis in the number of those diseases which at times may be associated with a positive Wassermann reaction.

SUMMARY AND CONCLUSION

1. A patient suffering from infectious mononucleosis presented a clinical picture similar to that of pre-eruptive and later secondary syphilis.

2. A four plus Wassermann and Kahn reaction and a maculopapular rash increased the differential diagnostic difficulties.

3. The underlying serological mechanism is discussed and the opinion is expressed that a proper evaluation of clinical and serological findings may be helpful in recognizing similar clinical manifestations.

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THE RELATION OF TRAUMA TO LEUKEMIA*

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While the cause of leukemia is unknown, there can be no doubt that certain factors play an important rôle in developing the symptoms of the disease. This presumes that the condition may exist in a latent or asymptomatic state. Cases of this type have been encountered by us from time to time and will be the subject of a future paper. Among the factors resulting in the development of symptoms of leukemia are infection, hemorrhage and trauma. That infection can cause marked stimulation of myeloid activity has been repeatedly demonstrated by the leukemoid blood pictures seen in some infections. Simon¹ reports a case with compound fracture of the right ankle where repeated blood counts taken for three weeks after fracture showed no leukemia. About four weeks after the fracture, cellulitis developed and the blood count showed 50,000 white blood cells with the presence of normoblasts and megaloblasts in addition to myelocytes. Following subsidence of the infection, the blood picture returned to normal. The purpose of this paper is to present the important relationship of the traumatic factor to the development of more or less severe symptoms of the underlying leukemic state. The following cases are illustrative of the importance of recognizing this apparent etiologic factor. With the exception of Case 3, all of the others were subjects of legal

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action and testimony had to be given as to the relation of trauma to the leukemia.

Case 1. R. C., well developed, robust male, aged 21. Occupation, carpenter. Had never consulted a physician before and did not remember being sick before the present illness. He was entirely well before September 14, 1932, when he was struck on the right shin by a shovel, causing laceration of the skin. The next day there was evidence of infection and swelling and the leg was treated by a local physician. Because of the infection he did not return to work. About a week after the injury glands became palpable in the patient's right groin and later in the left groin, axillae and neck and fever appeared. On September 26 the patient developed pain in the right lower quadrant of the abdomen and was sent to the hospital with a diagnosis of appendicitis. At the hospital, physical examination showed, in addition to the enlarged lymph glands, enlargement of the liver and spleen. The blood count showed a moderate anemia, the white blood count was 136,000, and differential cell count showed the majority of the cells to be myeloblasts and myelocytes. Nucleated red blood cells were also found in the smear. The patient died on October 5th, three weeks after receiving the injury. Autopsy verified the diagnosis of acute myeloid leukemia.

Case 2. T. W., white male, aged 63, had been in good health and working regularly as a carpenter and deck builder for the past 33 years. On April 17, 1936, while at work driving piles in the construction of a sewer, a heavy piling fell on his right foot and ankle, causing immediate pain, swelling and discoloration. X-ray taken that day showed a fracture at the ankle. About three weeks after the injury, while still receiving treatment for the injury, the patient noticed small hemorrhages in the skin of his right leg, and later, over his entire body. About six weeks after the injury, the patient noticed swelling of the glands in the right inguinal region, and later, swelling of the glands in other locations. Because of the progressively increasing weakness which prevented him from being up and around, he entered a hospital where a blood count showed lymphatic leukemia. The patient at that time had 85,000 white blood cells which were chiefly lymphocytes.

Case 3. M. J., white male, aged 73, was admitted to the hospital, March 16, 1937, with a history that five days prior to admission he had fallen and hurt his left hip. X-ray taken at the hospital showed a fracture of the neck of the left femur, in good position. The leg was put up in a cast and the patient apparently was comfortable and doing well. About two weeks after admission to the hospital, it was noticed that the patient was developing a waxy pallor and appeared very anemic. A blood count showed 30 per cent hemoglobin, 1,110,000 red blood cells, 6,150 white blood cells of which 26 per cent were immature

cells of the myeloid series, including 11 per cent of myeloblasts. On the basis of the blood count, a diagnosis of myeloid leukemia in the leukopenic phase was made. The patient became progressively worse and died about a month after admission to the hospital. Autopsy confirmed the diagnosis of myeloid leukemia.

The relationship of trauma to leukemia has been recognized as far back as 1845 when Virchow² reported a case of myeloid leukemia following injury to the bones. Wallace³, in 1855, likewise reported leukemia following trauma to the spleen. Leukemia has also been reported as resulting from a general concussion. More recently, cases of leukemia have been noted following injury to the soft parts, complicated by infection or hemorrhage. Lewson⁴, in 1930, collected 40 cases from the literature in which trauma was followed by leukemia.

From a review of the literature and the additional cases presented here, we must concede that symptoms of leukemia may follow shortly after trauma. There seems to be, however, some difference of opinion with respect to the exact nature of the relationship of the trauma to the development of the leukemic symptoms.

Some observers^{5,6,7} consider the trauma as the etiologic factor which is responsible for the development of the leukemia. Neumann⁸ believes that the trauma initiates or starts the leukemia in persons with a pre-existing tendency to the disease. Others^{9,10} feel that the trauma is the direct aggravating or exciting factor in the development of the leukemia.

In the majority of cases in the literature and in those of this series, the patient had apparently been in normal health and had been working or following his usual routine up to the time of the injury and there had been no blood examination prior to the development of leukemic symptoms. It is important to note in this connection that in the case reported by Greiwe¹¹, death occurred eleven days following trauma in a patient with a pre-existing leukemia.

The time elapsing following the trauma before the recognition of the leukemia varies for several reasons. In some cases, as in the Case 3 of this series, injury causes so intense a stimulation to the hematopoietic organs that the leukemia assumes an acute form and is diagnosed within a few weeks of the trauma, while in others (Case 2), the leukemic process assumes a more chronic course. Patients in this class occasionally return to work and then gradually notice progressive weakness or fail to respond to ordinary treatment directed to the injury. As a result of failure to recuperate or because of the appearance of symptoms of anemia, these patients are eventually referred to the hospital where a thorough examination is first made. Then the pallor, enlarged lymph nodes and spleen and typical blood picture are first noted. For this reason, the statement that leukemia follows three weeks to five years after injury, must be considered of doubtful importance.

While most of the cases in the literature are of myeloid leukemia, nevertheless cases of lymphatic leukemia are not unusual^{9,12}, one case in this series being lymphatic leukemia.

The importance of this problem has been forced upon us by being called before the compensation bureau to discuss such cases from the medico-legal standpoint. As a result of opposing testimony by insurance companies and claimants for disability, it has been a great responsibility to evaluate a definite relationship between trauma and leukemia and the important recognition of the latent form of leukemia. In some cases disability follows immediately after injury, whereas in others this may appear much later. Considerable doubt must be expressed as to cases developing possibly six months after injury. Such cases must be considered as bearing no relationship to the injury. Cases which develop leukemia shortly after the trauma must be considered as bearing a definite relationship between the trauma and the leukemic manifestations. In this connection, Liniger¹³ sets up the following criteria for associating any trauma with the subsequent development of leukemic manifestations:

1. The injured individual must have been well and feeling capable of work up to the time of the accident.

2. A suitably severe accident must have taken place, with the essential objective and subjective signs of injury.

3. The time at which the development of sickness starts and

that of the accident must bear a relation, that is, there must be bridging symptoms.

In our opinion, we must consider that the injury results in an aggravation of a pre-existing or asymptomatic leukemic state rather than as a direct etiological factor. We feel that the injury, whether traumatizing the bone or spleen directly or through stimulation of hematopoiesis through infection or hemorrhage, upsets the fine hematopoietic balance which has maintained the leukemia in its latent state and causes a more rapid progression of the process with development of frank symptoms of leukemia. It is our opinion that this type of stimulation of a latent or potential or preclinical leukemic hematopoietic system results in the development of the definite clinical leukemic picture.

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A PROBLEM IN BLOOD GROUPING*

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This report deals with a blood which presented an interesting and, actually, a not unusual problem in blood grouping. individual (S. G.) involved is a professional blood donor who had been placed in group B by several hospital and commercial donor bureau laboratories and who had donated on several occasions the usual amounts of blood (500 cc.), to patients of group B with no untoward results. The same donor, however, was found in several other instances to have been incompatible when tested with other patients of group B and was therefore rejected. When S. G. applied to the Donor Bureau of the Blood Transfusion Betterment Association, his blood was found to contain the factor B, but it was observed that his cells also reacted weakly but distinctly with potent grouping sera of group B (anti-A). Consequently the donor was placed in group AB. Subsequent investigation has shown that the factor A in this blood was considerably weaker even than the weakly reactive A of sub-group A^2 .

The data to be presented will show that unless very potent agglutinating sera are employed the factor A in such a blood is readily missed, and the blood incorrectly diagnosed as belonging to group B.

Furthermore, our investigations have shown that this blood presents still another difficulty, since it could readily be shown to contain an atypical agglutinin specific for the more sensitive bloods of sub-group A¹. This atypical agglutinin, identical with the anti-A¹ described by Landsteiner and Levine¹, reacted on about 80 per cent of groups A and AB cells (i.e., A¹ or A¹B) at

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20°C., or even at 25°C. and indeed more intensely than the iso-agglutination effect of unselected group B sera on this donor's cells. However, at 37° this reaction was almost entirely absent.

Two sorts of tests were performed in studying this blood: (1) the action of numerous sera of group B selected at random on the A²B cells of this donor, S. G. and on two other cells of group A (representative A¹ and A² cells). (2) the action of the specific anti-A¹ agglutinin in serum S. G. on cells of groups A and AB.

The routine tests were put up in small test tubes to which were added 1 capillary drop of serum, 2 capillary drops of 2 per cent washed cell suspension, and 1 drop of physiological saline. Readings were made as indicated, either

TABLE 1

ACTION OF GROUP B SERA (ANTI-A) ON CELLS OF S. G., A² AND A¹ BLOOD CELL
SUSPENSIONS

SERA OF GROUP B	S. G. (A ² B)	P. I (A ²)	P. B. (A1)
310	+±	++++	++++
334	+	+++	++++
393	±	+±	++++
407	tr.	+±	++++
325	0	+	+±
369	0	+	++
401	0	+	++±
Donor bureau	+±	++++	++++

Readings were made after the tests stood for 2 hours at room temperature.

after centrifuging at low speed for one minute and resuspending the sedimented cells, or else after the tests stood for one hour or longer at 20°, 37°C., or room temperature. For comparative purposes a few tests were also made on the open slide using a 10 per cent suspension of cells. In all cases, readings weak or negative to the naked eye were checked microscopically by withdrawing a drop of the mixture onto a slide by means of a thin glass rod.

A few of the numerous experiments performed which illustrate the characteristics of this blood are given below in the several tables:

It is obvious from the results in table 1, that the blood S. G. would be incorrectly grouped unless potent sera are employed. Although cells of sub-group A² are weakly agglutinated by some B sera, nevertheless our investigation shows that in tests of 28

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unselected group B sera not one was found which failed altogether to agglutinate typical A² cells (P. L.). With regard to the action of these 28 sera on cells S. G., however, 6 failed to show any degree of clumping, 4 gave only traces of agglutination, 8 showed very weak agglutination (less than +) and 10 gave weak but distinct reactions.

Similar tests kept at 37°C. or read after centrifuging and resuspending the sedimented cells showed practically identical results, the reactions being, on the whole, however, uniformly very slightly weaker in the tests kept at 37°C. The reactions

TABLE 2a ACTION AT 20°C. OF SERUM S. G. ON CELLS OF GROUP A

	SUB-GRO	OUP A2				SUB-GE	OUP A1		
P. L.	A, C.	B. Z.	395	301	314	321	343	350	351
0	0	0	0	+	+	+±	+±	+	+

TABLE 2B

ACTION AT 20°C. OF SERUM S. G. ON CELLS OF GROUP A B

SUB-GROU	P A2B		SUB-GROU	UP A1B	
S. G.	373	304	317	336	372
0	0	+	++	+	+

were also somewhat weaker in tests done on the open slide with heavier cell suspensions.

The study of the serum of S. G. revealed the atypical agglutinin specific for cells of sub-group A¹ of groups A and A B.

The action of the atypical agglutinin in serum S. G., as was to be expected, is heat labile, so that if tests are made at 37°C., only questionable traces of agglutination are observed. This fact is to be contrasted with the above-mentioned heat stability (at 37°) of the weak isoagglutination of those group B sera which are sufficiently potent to react on cells S. G.

We have since found a number of other bloods of group AB with an equally poorly agglutinable A factor, several of these in professional blood donors who like S. G. were wrongly diagnosed

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as B and at least one donated blood to group B individuals.* As far as we can ascertain, transfusions of AB blood into B donors have been uneventful in so far as immediate symptoms are concerned, but there is reason to believe that such blood does not survive for a long time in the recipient's circulation.† Some of these AB bloods referred to, differ from blood S. G. in that their sera do not contain the atypical agglutinin, and therefore, their correct diagnosis as AB presents no special difficulty provided that potent grouping sera are employed.

After the donor S. G. was grouped as an AB at the Blood Transfusion Betterment Association, he was sent out 10 times to donate blood, but in 2 instances he was rejected for reasons unknown to us. If one may speculate, it is possible that the grouping of S. G. was checked at the hospital and incorrectly grouped as B, or the atypical agglutinin in serum S. G. was found to act on the patient's cells (A¹B). The latter alternative does not seem likely if one assumes that in the direct matching it is only ascertained that the patient's serum does not act on donor's cells, and not the reverse. If only the one cross-test is done it is obvious that the majority of A B recipients (of sub-group A¹B) would receive injections of the atypical agglutinin which is, however, practically inactive at 37°C. There is certainly no reason to believe that this has harmful effects.‡ On the other hand, as noted above, it is likely that the blood injected into B recipients would not long survive, even if no immediate acute symptoms are encountered. This might be determined experimentally by actual transfusions, but to do so deliberately would involve unjustifiable human experimentation.

† Cf. Burnham² and Grove and Crum³.

^{*} This donor was a physician connected with a large university hospital.

[‡] The situation is not quite the same if S. G. were the patient. For in this case, the direct test would exclude the more frequent donors of sub-group A¹B since serum S. G. acts to a moderate degree on A¹B at 25°C. Even if this heat-labile reaction were missed, especially in the hands of inexperienced workers, the resulting transfusion would probably be uneventful since the atypical agglutinin in serum S. G. is practically inactive at body temperature.

In conclusion it is urged that only very potent grouping sera be employed.⁴ In view of our experience with blood S. G., it is suggested that anti-A sera of group B be standardized by action on blood of sub-group A². Such bloods are readily detected in an examination of a number of A bloods tested with fresh B or O sera. The two sorts of A's are then readily selected since some fresh sera will hemolyze cells of sub-group A¹ and agglutinate cells of A² without hemolysis. Without resorting to hemolysis, A² bloods can be selected since they are far less intensely agglutinated by sera O or B.

With regard to the specific problem of S. G. as a donor for AB patients, it is our opinion that he can be used safely for any patient of group A B (including sub-group A¹) in spite of his atypical agglutinin.

It is intended to describe in greater detail this and still other instances of irregular isoagglutination reactions which have been called to our attention by workers in several hospitals.

Our recent survey of blood transfusion in the United States (5) revealed altogether too many avoidable transfusion accidents attributable at least in large measure to errors in blood grouping and cross-matching tests. The performance of these procedures by properly trained workers and the exclusive use of potent grouping sera are essential for the prevention of transfusion accidents.

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A SIMPLIFIED METHOD FOR THE PREPARATION OF AN ANTIGEN FOR USE IN THE COMPLEMENT FIXATION TEST FOR SYPHILIS*

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Among the numerous antigens used in the complement fixation tests for syphilis the more sensitive result from the extraction of dried tissue, first with acetone and ether, and then with alcohol. The preliminary procedure is relatively brief, but the alcohol extraction is generally prolonged. Beef heart—pooled, dried and powdered—when freed of its anticomplementary properties and then extracted, is the tissue which has been found to give the most uniformly satisfactory antigens containing a high concentration of the desirable lipoids. The exact chemical constitution of the active component is still under discussion, but the weight of evidence at present available points to the lecithin fraction as the one for which the syphilitic reagin has the greatest avidity. It has been learned, furthermore, that cholesterol, in proper concentration, enhances the sensitivity of antigens prepared by the extraction of beef heart.

Variations in the technical procedures in current use for the preparation of antigens have to do with increase in the number, or decrease in the time, of the preliminary extractions, differences in the duration of the alcoholic extraction, subsequent concentration of the extracted material, or in the amount of cholesterol added. All are satisfactory for the production of antigens of proven usefulness in the complement fixation test for syphilis.

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The valid criticism which may be made of these antigens is that their preparation requires several days. Alternate shorter methods usually involve the use of special apparatus and extraction with boiling alcohol. It has been shown that the preliminary extraction with ether and acetone can be satisfactorily carried out at room temperature and that prolonged extraction is not a requisite. Such rapid methods often produced antigens as sensitive as those made by the more prolonged extraction procedures, but the result could not be predicted and the products were not uniformly valuable.

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A series of experiments has been undertaken with the primary purpose of finding a dependable and rapid method for preparing antigens of high sensitivity. Economy of time is always, of course, a secondary consideration unless equal or better results can be obtained by a rapid method. An antigen of high sensitiveness enhances the practical usefulness of the simplified complement fixation technic described in a separate publication².

MATERIALS AND METHODS

Pooled, dried, and powdered beef heart (Difco) was used. In earlier experiments the basic procedure consisted of a preliminary washing of the powdered beef heart with acetone or ether, or both. These washings were made for varying periods of time up to 1 hour, with and without the aid of heat. The powder was freed of the wash solutions, dried, and then extracted for varying periods of time, usually with boiling alcohol. Where the extraction in boiling alcohol was carried out for longer than 1 hour a reflux condenser was used. Some of the extractions were done serially on the same sample of beef heart with several fresh portions of alcohol, after which these various alcoholic extracts were pooled and concentrated. Varying degrees of concentration of the extracted material were made by evaporating the solutions on the water bath and varying degrees of fortification of the antigens with the acetone insoluble lipoids and cholesterol were tried. After preparation, all these experimental antigens were compared under identical conditions with antigens made by the methods described by Kolmer and by Kahn. The method of comparison used was the antigen titration technic described by Hooker³ and recommended by Boerner and Lukens.4 All these tests were made with the same positive serum, so diluted that the maximum dilution would correspond with a minimal-almost undetectable-amount of reagin. Similar titrations of the inherent hemolytic and anticomplementary properties were made and compared with other antigens taken for standards, and with the alcohol used in the extraction.

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Extracts made with boiling alcohol, on cooling, formed a white precipitate which becomes considerably greater in amount if the extract is cooled to 6° to 8° in the refrigerator. This material when separated from the supernatant fluid is only slightly soluble in cold alcohol, and when partially dissolved in alcohol the resultant solution is highly anticomplementary. The supernatant fluid after the precipitate had been removed proved to be somewhat increased in sensitivity. However, none of the products resulting from this series of experiments was considered to show marked improvement over those prepared by the older methods, although the latter produce slightly less sensitive extracts.

It was then reasoned that since the efficacy of the antigens used in the complement fixation tests for syphilis seems to depend on their lipoid content then current biochemical methods employed for the quantitative extraction of lipoids from biologic materials in analytical procedures might be utilized for our purposes. Bloor's method employs a 3:1 alcohol and ether mixture. Failure to obtain quantitative extraction with this method was shown by Boyd⁵ to be the result of insufficient dilution of the material in the solvent. In the case of blood serum, quantitative lipoid extraction is complete in a few minutes if the volume of extracting fluid is at least 20 times that of the material to be extracted. With more granular material even larger volumes of extracting fluid are required—up to 30 to 35 times the volume of the material to be extracted. Such relationship between the volume of solvent and the material to be extracted was employed in making an antigen. The extracting reagent was the 3:1 alcohol-ether mixture of Bloor. Others have employed alcohol-ether mixtures but found that the resultant antigen solution possessed hemolytic properties which rendered it unsuitable for use in the complement fixation test for syphilis.6

After the preparation of the antigen, it was purified by precipitation in the ice box at 6 to 8°C., and then fortified with concentrations of cholesterol varying from 0.1 to 1.0 per cent. Similar portions of fortified and unfortified antigen were then titrated and compared under identical conditions, with the Kolmer and Kahn standard antigens. The anticomplementary and hemolytic properties were compared, likewise, with one another and with the standards. The unfortified antigens were as sensitive as the standard antigens and those containing cholesterol were even more sensitive than the new unsensitized antigens which were no more anticomplementary than the standard antigens and showed only slightly more anticomplementary action than the alcohol in which they were dissolved. There was little hemolytic activity. Perhaps the reason our extracts lacked hemolytic activity and the anticomplementary effect of the other ethereal extracts rests in the fact that after preparation of the alcohol-ether extract it was concentrated by evaporation until the final amount was only five times that of the original amount of beef heart used. This concentration had the effect of driving off any remaining ether so that in the end, the antigenic lipoids were dissolved in alcohol alone.

The experiments were then repeated. In one, 25 grams of the beef heart

were extracted for 1 hour with 750 cc. of the alcohol-ether mixture. Simultaneously, 10 gram portions were extracted, each with 300 cc. of the alcohol-ether mixture for 15, 30, 45, and 60 minutes. After extraction, the 25 gram extract was concentrated with heat, aided by partial vacuum, to five times the amount of beef heart extracted (125 cc.); each of the 10 gram batches was concentrated to 50 cc. Each resultant antigen was divided into two parts, one-half was placed in the ice box at 6 to 8°C. for 1 hour, and the other half allowed to stand at room temperature overnight. The precipitate which formed in each was filtered out and the antigens stored at room temperature. A portion of each antigen thus prepared was fortified with cholesterol, after which they were all compared under the same conditions.

The result was that every one of the antigens of this series was more sensitive than the control antigens and lacked noteworthy anticomplementary properties or hemolytic activity.

With this encouragement we then proceeded to study the possible influence of variations in the different steps in an effort to learn the optimum conditions for producing antigens under these promising circumstances. Beyond five minutes, the time of extraction caused no significant increase in sensitivity; the total solids were surprisingly constant regardless of whether or not the material was extracted for 5, 10, 15, 30, or 60 minutes. Antigens precipitated in the ice box were consistently more sensitive than those precipitated at room temperature overnight. Those antigens precipitated in the ice box remain practically clear after a month's storage at room temperature, whereas further precipitation occurs in those not placed in the refrigerator. The precipitates that form in the ice box and at room temperature behave quite alike with regard to their solubilities. They are partially soluble in cold alcohol, freely soluble in boiling alcohol, completely soluble in ether, but insoluble in acetone. After solution in ether, the addition of either alcohol or acetone or magnesium chloride causes a precipitate to form. This is reminiscent of the fact that the addition of ether to antigens not purified by cooling in the ice box results in the formation of a precipitate. Antigens from which the precipitate formed at 6 to 8° has been removed remain clear after the addition of ether.

The above experiments were repeated using 95 per cent alcohol. These extracts were compared with those made with absolute alcohol and with the standard Kolmer and Kahn antigens. Those made with 95 per cent alcohol were practically as sensitive as those made with absolute alcohol. Comparative tests show that the various extracts can be concentrated without loss in sensitivity by boiling, with or without the use of negative pressure; the vacuum pump merely hastens evaporation. The absolute alcohol extracts are much more easily concentrated than are those made with 95 per cent alcohol when a partial vacuum is not employed; for this reason absolute alcohol is to be preferred.

The method described in detail below permits the rapid preparation of a highly sensitive antigen from dried beef heart powder

and does not involve preliminary extraction with acetone and ether. The entire preparation of the antigen can be accomplished in $2\frac{1}{2}$ hours.

SIMPLIFIED TECHNIC FOR THE PREPARATION OF THE ANTIGEN SOLUTION

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1. Alcohol-ether mixture, which is prepared by mixing three parts of either 95 per cent or absolute alcohol (neither requires redistillation for use, if a good grade of alcohol is used), with one part of C. P. ether.

2. Powdered, dried beef heart (Difco).

Method:

 Weight accurately the amount of beef heart powder required to make the volume of antigen to be prepared. The amount of finished antigen will be five times that of beef heart powder used. Thus, if 125 cc. of finished antigen is wanted, the amount of beef heart powder to be weighed out

will be 25 grams.

2. Transfer the weighed beef heart powder to a suitable vessel for extraction. Add a volume of the alcohol-ether mixture equal to 30 times the weight of the beef heart powder used. If 25 grams of beef heart are used, then 750 cc. is the necessary amount. It is best to do the extraction in a 1-liter Erlenmeyer flask and stopper it during the extraction. The extracting fluid should be prepared after the beef heart powder is weighed to prevent undue loss of the highly volatile ether. The flask is agitated thoroughly at least every five minutes. Extraction is satisfactory in five minutes; the results are possibly more nearly uniform, however, if the extraction time is lengthened to 30 minutes.

3. Filter. Any good grade filter paper is satisfactory.

4. Place the filtrate in a water bath and concentrate it by boiling until the total volume is five times the weight of the original beef heart powder used. If the concentration happens to be carried too far, simply add sufficient alcohol to make up the deficiency. The evaporation is greatly accelerated by the use of a partial vacuum, though the result is not changed thereby. After the evaporation procedure is completed the fluid-antigen should be entirely free from any odor of ether.

5. Transfer the resultant concentrated antigen solution to a small flask and place it in the ice box at 6 to 8°C. At the same time place in the ice box a small funnel with filter and a suitable receptacle for the final purified antigen solution. After at least a full hour in the refrigerator, the concentrated antigen solution, which should now contain a copious white precipitate, is filtered through the cold filter into cold container.

One hour refrigeration precipitates enough inert material so that no significant further precipitation occurs when the antigen is kept at room temperature. Where time is not a factor, longer periods of refrigeration (overnight) remove more completely the inert material. This longer period of chilling does not affect the antigenic properties of the solution, and may be desirable because further precipitation is not likely to occur. Regardless of time of refrigeration the filtered antigen should be clear. On standing a small amount of precipitate may form which may be ignored.

- 6. The filtered antigen is now measured and the desired amount of cholesterol is added. It is our present custom to use 0.4 per cent cholesterol, but any other quantity may be used whether the individual serologist prefers more or less sensitivity. Add the cholesterol directly to the alcoholic antigen solution and dissolve by heating in a water bath at 56 to 58°C. This is the final product and should be kept at room temperature. It should remain almost entirely clear for at least a month.
- 7. Before use, the optimum antigenic dose is determined using the method recommended by Boerner and Lukens, which is based on the method described by Hooker. Details of the antigen titration by this method are described in connection with a simplified complement fixation test for syphilis², and elsewhere³. 4, 7, 8.

Examples of the specificity, antigenic sensitivity, anticomplementary and hemolytic properties of antigens prepared by this method are shown below in some representative comparative titrations. The simplified technic of Boerner and Lukens was used in these tests.

Antigen Titration

			Ar	itigen T	itration				
SERUM ANTIGEN	1:400	1:600	1:800	1:1200	1:1600	1:2400	1:3200	1:4800	1:640
			Kolme	r antige	n numb	er 8			
0.1	4	4	4	4	4	4	4	3	2
0.05	3	4	4	4	4	4	4	3	3
0.025	R	+	土	1	2	2	3	1	1
0.0125	-	_	_	-	-	-	-	_	
	Simpli	ified ant	igen nu	mber 3	with no	added o	holeste	rol	
0.1	4	4	4	4	4	2	2	1	1
0.05	4	4	4	4	4	3	2	1	1
0.025	1	2	1	3	2	1	2	±	R
0.0125	_	-	_	_	_	_	_	_	_

Sin	nplified	antigen	number	3 with	0.2 per	cent ch	olestero	l added	
0.1	4	4	4	4	4	4	4	4	4
0.05	4	4	4	4	4	4	4	4	4
0.025	3	4	4	4	4	4	4	3	2
0.0125	±	-	+	-	1	1	1	±	±
Sim	plified	antigen	number	3 with	0.4 per	cent ch	olestero	l added	
0.1	4	4	4	4	4	4	4	3	3
0.05	4	4	4	4	4	4	4	3	3
0.025	4	4	4	4	4	4	4	2	3
0.0125	±	+	±	1	1	1	1	±	1
Sim	plified	antigen	number	3 with	0.6 per	cent ch	olestero	l added	
0.1	4	4	4	4	4	4	4	2	1
0.05	4	4	4	4	4	4	4	2	1
0.025	4	4	4	4	4	4	4	2	+
0.0125	3	3	3	2	4	3	R	R	_

None of these antigens was hemolytic in a dilution of 1:12. The antigens were but slightly more anti-complementary than the alcohol in which they were dissolved.

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The same positive serum was used in all the tests. All tests were incubated at 6 to 8°C. for 16–18 hours, then placed in the water bath at 37° for 10 minutes before the sensitized cells were added. After the addition of the sensitized cells they were kept in the water bath at 37°C. for a full hour.

SUMMARY

1. A rapid, simple method for the preparation of antigen for use in the complement fixation test for syphilis is described.

2. The antigen is sensitive to a superior degree and quite specific in the complement fixation test for syphilis.

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BASOPHILIC AGGREGATIONS IN THE BLOOD OF THE NEWLY BORN*

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(A) LABORATORY ANIMALS

(B) HUMANS

CAREY P. McCORD†

AND

WILLIAM R. BRADLEY!

In the practical application of the basophilic aggregation test as an aid in the diagnosis of lead absorption and lead poisoning, always it has been recognized that this procedure is in no way specific as an indication of lead action. Increased numbers of basophilic aggregations are but proof of one response of the bone marrow to a variety of physiologic and pathologic stimuli. As a consequence, aggregations in excess of those numbers regarded as usual for healthy adults may be expected in diverse physiologic and pathologic states including the new born, anemias unassociated with bone marrow hypofunction and in intoxications produced by such materials as benzine, bismuth and lead.

In the adult healthy human the percentage of basophilic aggregations is usually below 1.0 per cent, frequently as high as 1.5 per cent but rarely as high as 2.0 per cent. Within this normal range, undoubtedly many influences bring about fluctuations daily and over longer periods. These variations within normal limits may be of significance, but in the use of this testing procedure in connection with the diagnosis of lead poisoning or lead absorption no consideration is usually attached to findings until the number of basophilic aggregations rises above 1.5 or 2.0 per cent.

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Department of Health, Detroit.

It is believed that these aggregations, as seen under the microscope, 'after staining, represent a protoplasmic alteration not observable as such in erythrocytes of the circulating blood and thus may be regarded as laudable artifacts. The number of basophilic aggregations in any properly made preparation exceeds the number of reticulocytes since the former represents the sum total of basophilic-containing erythrocytes such as pre-formed stippled cells, polychromatophilic cells and nucleated red cells when these last mentioned cells contain basophilic substances.

When lead poisoning is produced in laboratory animals, particularly albino rats, some confusion may arise because of large numbers of basophilic aggregations in some control animals. In one instance this was traced to the accidental occurrence of trypanosomal infection, which condition, in our experience, produces in albino rats very high percentages of basophilic aggregations. In other series of experiments unexpected numbers of basophilic aggregations and stippled cells were found in all animals prior to the introduction of any lead compounds. On investigation it was disclosed that all such animals, while apparently of adult age, were, in fact, young animals with respect to their blood picture.

Since it is known that the blood of the fetus and the newly-born of many animals contains immature cell forms, it was determined to establish percentages of basophilic aggregations at short intervals after birth in order to fix that time at which substantially constant numbers of basophilic aggregations begin to exist. Also, as this test is often applied to young children under conditions of suspected lead poisoning, it was deemed desirable to parallel animal studies with similar examinations of the blood of premature and full term infants, continuing such tests until normal levels were established. The results from this two-phased study led to this report.

BACKGROUND LITERATURE

Pertinent to this present publication is an extensive literature dealing with the occurrence, source, significance and characteristics of immature erythrocytes including numerous discussions of basophilic substance in erythrocytes as one indication of cellular immaturity. Some items in this literature are concerned with unripened erythrocytes in various anemias, hemorrhages, infectious diseases and intoxications in the embryo and in the newborn. For reasons of space these fundamental publications may be neither reviewed nor even extensively cited, but as leads to background literature, a few key publications are indicated at the end of this article.

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(A) BASOPHILIC CONTAINING ERYTHROCYTES IN YOUNG AND MATURE LABORATORY ANIMALS

In this section of the study there have been utilized 350 albino rats (*Mus norvegicus albinus*), 50 mature rabbits, 25 mature guinea pigs and 25 mature dogs.

Chief activities centered about the albino rat groups on which examinations were made from birth to complete maturity as shown in table 1. Total red and white blood cell counts were made together with determinations of the hemoglobin, nucleated erythrocytes, polychromatophilic, stippled and basophilic aggregations cells. Conventional techniques were employed in determining the erythrocytes and leukocytes. Hemoglobin was determined by the Sahli method (14.5 grams = 100 per cent). The procedure used in the basophilic aggregation test, in establishing polychromasia and in demonstrating preformed stippled cells was that described by Hyler and Bradley¹⁰.

These rats were maintained on a standard rat diet containing ground corn, wheat, middlings, soy bean oil meal, meat scraps, wheat bran, linseed oil meal, bone meal, calcium, salt and cod liver oil. Lettuce and carrots were provided twice weekly. So far as determinable, these rats were healthy animals, in the best of condition, closely adhering to age-weight curves long established for growing rats in this laboratory. Absence from upper respiratory infection, middle ear infection and chronic ulcerative cecitis was determined. Many animals were submitted to autopsy as a further control measure.

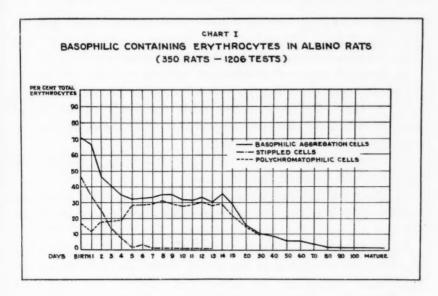
No attempt was made to differentiate the sex of rats in the age group under 15 days. Equal numbers of male and female animals were used in subsequent age groups. In examining the blood of rats only a few hours or days old, it was necessary to sacrifice the animals in order to obtain sufficient blood for the various tests. Five examinations of blood from the rat fetus disclosed basophilic aggregations of a high concentration similar to that of the rat at birth. Diameters of blood cells were measured against a filar micrometer.

A summary of accrued data is shown in table 1. Findings with reference to basophilic containing red blood cells are likewise shown in chart 1. While these figures as presented represent the mean or average, it is here noted that deviation from such mean is not significant.

TABLE 1
SUMMARY OF DATA ON YOUNG AND MATURE ALBINO RATS

AGE	NUMBER OF ANI- MALS (TOTAL 350 —5 PETUBES)	WEIGHT	HEMOGLOBIN	AVERAGE	AVERAGE LEUKO- CYTES (PER CU. MM.)	DIAMETERS OF ERTTHROCYTES	BASOPHILIC AGGREGATION CELLS (PER CENT TOTAL B.B.C.)*	POLYCHROMA- TOPHILIC CELLS (PER CENT TOTAL R.B.C.)*	STIPPLED CELLS (PERCENT TOTAL R.B.C.)*	NUCLEATED ENTHHOCTIES (PER CENT TOTAL
		grams	per cent	millions per cu. mm.		microns				
2 hours	6	4.9	92.0	2.7	2,592	10.2	71.7	16.0	46.3	0.40
12 hours	8	5.0	88.0	2.8	2,894	10.0	70.5	18.5	44.1	0.80
1 day	6	5.7	82.8	2.9	3,503	8.7	66.8	12.4	34.8	0.20
2 days	8	7.3	74.5	3.0	3,000	8.7	46.2	17.3	24.4	0.05
3 days	6	6.7	77.4	2.4	3,035	8.4	40.8	18.6	14.5	0.07
4 days	7	7.8	72.6	2.8	2,733	8.6	34.1	18.9	7.6	0.00
5 days	10	7.3	71.0	2.9	3,094	8.3	31.5	27.9	1.3	0.00
6 days	7	10.7	69.7	3.1	3,001	8.2	32.4	28.3	3.0	0.02
7 days	6	10.8	67.0	3.1	2,908	8.2	33.7	29.0	0.8	0.01
8 days	6	16.6	55.0	3.1	3,150	8.0	35.9	32.5	1.0	0.08
9 days	12	17.5	62.8	3.3	3,546	8.0	35.4	29.5	0.5	0.00
10 days	12	19.5	63.5	3.8	3,778	8.0	32.2	27.6	0.7	0.00
11 days	10	16.2	70.0	4.0	4,148	8.0	31.8	28.2	0.06	0.00
12 days	10	18.8	70.6	4.0	4,151	8.0	33.5	30.5	0.0	0.00
13 days	10	25.7	70.6	4.2	3,966	7.9	30.4	27.7	0.02	0.00
14 days	10	29.9	66.4	4.3	3,527	7.9	35.0	28.1	0.02	0.00
15 days	10	24.6	54.0	4.3	3,941	7.9	29.0	22.0	0.06	0.00
20 days	10	28.8	58.5	5.2	6,273	7.8	15.8	15.0	0.0	0.00
30 days	10	67.5	68.8	5.9	6,766	7.1	10.3	9.3	0.0	0.00
40 days	10	102.9	74.8	6.1	10,030	6.9	9.8	9.0	0.0	0.00
50 days	10	122.3	78.0	6.7	8,031	6.9	5.4	4.9	0.0	0.00
65 days	10	126.7	83.8	7.6	8,527	6.9	5.2	5.0	0.0	0.00
75 days	10	168.2	90.8	7.0	9,575	6.9	4.3	4.0	0.0	0.00
80 days	10	174.4	89.0	7.0	10,081	6.9	2.4	1.8	0.0	0.00
85 days	10	177.2	90.0	7.5	10,207	6.9	2.8	1.2	0.0	0.00
90 days	6	192.3	89.3	6.7	8,933	6.9	3.1	1.9	0.0	0.00
95 days	6	226.0	96.0	8.8	8,557	6.9	2.4	1.4	0.0	0.00
100 days	6	231.0	94.2	9.2	8,950	6.9	2.3	1.0	0.0	0.00
Mature male	80	315.6	96.4	8.9	9,420	6.9	2.1	2.0	0.0	0.00
Mature fe- male	80	237.2	87.6	7.6	8,460	6.9	2.3	2.2	0.0	0.00
Mature fe- male and male	160	276.4	92.0	8.3	8,940	6.9	2.2	2.1	0.0	0.00

^{*} Note: These figures represent averages of percentages derived from cell universes of different orders. It is recognized that this procedure is open to criticism by statisticians. However, the order of error is not such as to vitiate the trend of results.



Basophilic aggregation tests were made on other normal, healthy, mature laboratory animals in an effort to establish the mean for normal variations. These results are now shown:

ANIMAL	NUMBER OF ANIMALS	AVERAGE PER CENT BASOPHILIC AGGRE- GATION CELLS
Rabbits	50	1.8
Guinea pigs	25	0.9
Dogs	25	0.6

COMMENT AND SUMMARY ON ANIMAL SERIES

- 1. At birth the albino rat presents a high percentage (71.7) of basophilic aggregation cells, 46.3 per cent pre-formed stippled cells and 16 per cent countable polychromatophilic cells.
- 2. The percentage of basophilic aggregation slowly diminishes but not until about the 80th day of life does this reduction approach that percentage (approximately 2.2) which characterizes the normal mature albino rat.
- 3. At no time is it possible accurately to enumerate polychromatophilic cells when stained with Romanowsky dyes. Such cells as are countable show an increase from 16.0 per cent

at birth to 32.5 per cent as a peak which is observable near the 8th day of life. Thereafter a diminution takes place so that from the 80th to the 100th day of life the percentage is below 2.0.

4. Large numbers of pre-formed stippled cells are present at birth (46.3 per cent) but by the 4th day the percentage is only 7.6. At the end of the 10th day of life practically all pre-formed stippled cells have disappeared and thereafter are not found except rarely and in trivial numbers.

5. No significant difference as to basophilic-containing red cells was observed between the two sexes.

6. The usual percentage of basophilic aggregations in mature rabbits, guinea pigs and dogs respectively was found to be 1.8, 0.9 and 0.6.

7. Manifestly investigations involving basophilic substance in erythrocytes as criteria of the action of extraneously introduced substances should not be carried out without due regard for physiologic changes extending in the case of the rat to approximately the 100th day of life.

(B) PREMATURE AND FULL TERM INFANTS

Ninety infants represented the human material upon which basophilic aggregation tests were carried out. Of these 20 were premature and 70 full term. In no known instance were tests performed on infants with any abnormality except as prematurity in itself may constitute an abnormality. Any anemia, diarrhea, jaundice or infection led to the elimination of data. However, minor skin diseases were not regarded as significant. Approximately equal numbers of male and female, colored and white infants were utilized in each respective age group. In procuring blood samples, the heel was the universal site in premature and smaller full term infants and the thumb in larger ones. Customarily blood samples were collected during sleep and at the same hour on each sampling day (10 to 12 a.m.). Apart from such details, the general preparation of material was the same as in the case of animals and as described in an earlier publication¹⁰.

In a series of tests on four infants, conducted over a 40 day period, each infant was examined daily during the first 10 days following birth, and thereafter at longer intervals (table 2). In two other series, extending over 40 days and 10 days respectively, such suitable infants were examined who had attained the proper age on those days designated for blood sampling (tables 3 and 4). Routinely complete blood counts and other related examinations were not carried out, but in the aggregate a large number were made in order to rule

TABLE 2

PER CENT BASOPHILIC AGGREGATIONS IN FOUR PREMATURE INFANTS

Successive tests made on same infant

NAME	Тн	MA	Kı	Bu
Sex	Female	Female	Male	Male
Race	Colored	White	White	Colored
Body Weight	2 lb. 10 os.	3 lb. 9 oz.	3 lb. 3 oz.	3 lb. 0 os.
days				
At birth	5.9	5.4	5.2	5.5
1	5.0	4.6	4.3	4.5
2	4.2	3.8	3.6	3.9
3	3.5	3.1	2.9	3.2
4	2.9	2.1	2.3	2.4
5	2.2	1.8	1.6	1.8
6	1.7	1.5	1.1	1.3
7	1.3	1.2	0.9	1.0
8	1.0	0.7	0.6	0.8
9	0.8	0.5	0.4	0.5
10	0.7	0.5	0.6	0.6
15	0.4	0.7	0.4	0.5
20	0.5	0.4	0.6	0.7
25	0.5	0.4	0.5	0.6
30	0.6	0.5	0.6	0.5
35	0.7	0.6	0.6	0.6
40	0.8	0.7	0.6	0.7

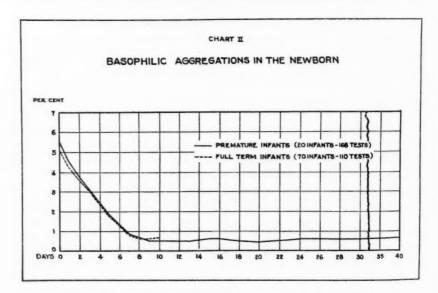
TABLE 3
PER CENT BASOPHILIC AGGREGATIONS BY AGE GROUPS
20 premature infants—168 tests

AGE	P	per cent basophilic aggregations (8 tests per age group)											PER CENT BASOPHILIC AGGREGATIONS (8 TESTS PER AGE GROUP)							
days					1															
At birth	5.1	5.8	5.0	5.9	5.2	5.6	5.8	5.8	5.5											
1	4.1	5.0	4.3	5.0	4.1	4.9	4.2	4.8	4.6											
2	3.4	3.1	4.5	3.5	4.2	3.4	4.2	3.7	3.8											
3	3.2	2.6	3.5	2.6	3.6	3.0	2.9	3.5	3.1											
4	2.5	1.8	2.8	2.0	2.9	2.6	2.0	2.8	2.4											
5	1.9	1.3	2.1	1.5	2.2	1.9	1.4	2.5	1.8											
6	1.4	1.1	1.5	1.0	1.7	1.2	0.8	1.9	1.3											
7	0.9	0.9	1.0	0.7	1.2	0.9	0.6	1.0	0.9											
8	0.7	0.7	0.7	0.5	1.0	0.7	0.5	0.7	0.7											
9	0.6	0.5	0.5	0.5	0.7	0.5	0.5	0.5	0.5											
10	0.5	0.5	0.7	0.3	0.7	0.3	0.5	0.5	0.5											
11	0.5	0.5	0.5	0.4	0.7	0.5	0.6	0.5	0.5											
12	0.4	0.5	0.5	0.5	0.7	0.5	0.4	0.6	0.5											
13	0.6	0.5	0.4	0.4	0.6	0.4	0.5	0.5	0.5											
14	0.5	0.5	0.6	0.6	0.6	0.4	0.3	0.5	0.5											
15	0.4	0.6	0.8	0.6	0.6	0.5	0.5	0.7	0.6											
20	0.6	0.5	0.6	0.5	0.4	0.5	0.5	0.5	0.5											
25	0.7	0.6	0.7	0.6	0.6	0.4	0.5	0.6	0.6											
30	0.6	0.5	0.5	0.4	0.8	0.5	0.6	0.5	0.5											
35	0.7	0.6	0.7	0.5	0.8	0.5	0.4	0.6	0.6											
40	0.9	0.7	0.7	0.6	0.9	0.7	0.5	0.5	0.7											

out suspected abnormalities such as might be determined through blood examinations.

TABLE 4
Per Cent Basophilic Aggregations by Age Groups
70 full term infants—110 tests

AGE		PER CENT BASOPHILIC AGGREGATIONS (10 TESTS PER AGE GROUP)										
days												
At birth	5.3	5.2	5.8	4.9	5.0	5.9	4.5	5.1	4.7	4.9	5.1	
1	3.8	3.9	5.0	4.3	4.3	4.5	4.1	3.9	4.8	4.5	4.3	
2	4.0	3.5	3.8	3.3	4.0	3.7	3.6	3.4	3.5	3.6	3.6	
3	2.6	2.6	3.5	3.4	3.2	3.0	3.3	3.0	2.4	2.6	3.0	
4	2.5	3.0	2.2	2.6	2.7	2.1	2.0	2.3	2.5	2.4	2.4	
5	1.7	1.8	1.1	1.3	2.2	1.5	1.4	2.3	2.1	1.9	1.7	
6	1.0	1.4	1.5	1.3	1.7	1.0	1.5	1.4	1.2	1.3	1.3	
7	0.7	0.5	0.8	1.0	0.9	0.7	0.5	1.1	0.8	0.7	0.8	
8	0.8	0.7	0.6	0.8	0.4	0.6	0.5	0.6	0.5	0.6	0.6	
9	0.5	0.8	0.6	0.5	0.6	0.5	0.4	0.7	0.8	0.6	0.6	
10	0.9	0.7	0.8	0.7	0.9	0.5	0.7	0.6	0.8	0.5	0.7	



Two hundred seventy eight basophilic aggregation counts were made. The resulting data has led to the tables 2, 3 and 4 and to chart 2. The same

opportunity for criticism indicated as a footnote following table 1 exists in connection with the method of presentation of these findings on infants.

COMMENT AND SUMMARY ON HUMAN INFANT SERIES

- 1. As in the case of laboratory animals, new-born infants present basophilic aggregations obtained from circulating blood in numbers and percentages in excess of adult, healthy individuals.
- 2. The maximum basophilic aggregation percentage which averages 5.3 was present during the first 24 hours after birth both in premature and full term infants. Unlike animals, a diminution to adult percentages rapidly takes place and usually at about the 8th day a normal level is reached.
- 3. Continuation of testing in premature infants to the end of the 40 days after birth indicates no substantial rise after normal percentages have been reached.

GENERAL SUMMARY

On a physiologic basis, both new-born animals and humans exhibit high percentages of erythrocytes containing basophilic substance (albino rats 71.7 per cent, infants 5.3 per cent).

In the case of infants this high percentage rapidly diminishes until near the 8th day of life when it approximates the percentage usual for human adults (0.5–2.0 per cent).

Occasion for the application of the basophilic aggregation test in instances of suspected lead poisoning in young children is not nullified on the assumption that children beyond ten days of age may continue to exhibit high percentages of these cells.

In the case of young rats, high percentages of erythrocytes containing basophilic substance persist until near the 100th day of life. This fact militates against their use in certain experimental work such as lead studies in which increased numbers of erythrocytes with a basophilic content are regarded as a criterion of lead action.

ACKNOWLEDGEMENT

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THE DIAGNOSIS OF TUBERCULOSIS IN 10-12 DAYS* BY GUINEA PIG INOCULATION

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In 1924, H. J. Corper¹ reviewed the various methods of guinea pig inoculation recommended in the preceding twenty years for laboratory diagnosis of tuberculosis, and came to the following conclusions:

1. We know no method which will hasten the development of tuberculosis in the guinea pig and be of practical value for giving earlier diagnostic information.

2. Years of experience with inoculation of tubercle bacilli in known and graded amounts into guinea pigs convinces one that such inoculations into various parts of the body can lead to only slight and relative, but not practical, differences in the rate of development of tuberculosis.

3. Recourse to methods, such as roentgen-ray exposure, injection of radioactive substances or other leucotoxic agents are based on erroneous observations, and do not hasten the development of tuberculosis in the guinea pig.

I now wish to report a method of guinea pig inoculation for the laboratory diagnosis of tuberculosis, which yields information in about one-third of the time usually required for such diagnosis. This method was discovered accidentally, in the course of experiments dealing with problems of natural immunity.

I had been trying to overcome the natural resistance of white mice to infection with human and bovine strains of tubercle bacilli, but without success. Normally, white mice are able to dispose quite rapidly of fairly large numbers of tubercle bacilli injected subcutaneously, and there is very little local tissue re-

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action produced by the bacilli. In my readings, I came upon an article by W. E. Guy and E. H. Kettle, entitled, "Silicosis and Miners Phthisis". These British investigators were trying to explain the well known high susceptibility of the silicotic lung to tuberculosis. They injected subcutaneously into white mice the colloidal sol of orthosilicic acid, Si(OH)4, followed with an injection into the same area of a suspension of tubercle bacilli, and found that in the "silicated" mice the injected bacilli did multiply. Their explanation was that the silicic acid injured the tissues, producing a coagulum which furnished a favorable nutrient medium for the tubercle bacilli.

I repeated this experiment. The sol of orthosilicic acid was prepared according to the method of W. E. Bullock and W. Cramer³ by treating a solution of sodium silicate with an excess of strong hydrochloric acid, dialysing the mixture against distilled water until all chlorides were removed, and filtering it through a Berkfeld filter.

My findings in a series of mice were disappointing. When light suspensions of bacilli were used, though each mouse received a dose of tubercle bacilli sufficient to infect several guinea pigs, I could find little, if any, convincing evidence of multiplication of the injected bacilli. There was no doubt that the bacilli disappeared from the tissues affected by the silicic acid considerably more slowly than in the controls. Heavy suspensions of tubercle bacilli, when combined with the toxic action of the silicic acid, produced much tissue destruction, but one would not venture to affirm that there was any pronounced increase in the number of the bacilli injected.

Quite different results were obtained with guinea pigs in similar experiments. When 1 cc. of the colloidal sol was injected subcutaneously and followed by 0.5 cc. of a very light suspension of tubercle bacilli, in a few days abscesses formed, containing large numbers of tubercle bacilli,—many times the number injected and definitely more than in the controls.

The sol of orthosilicic acid, while easy to inject, requires much time for its preparation, and is not uniform in its action. Its effectiveness apparently depends upon the nature of the dialysing bag used, a rather uncertain factor. Besides, upon standing it turns into a gel (Metasilicic acid—H₂SiO₃), and soon becomes too thick for injecting. I, therefore, experimented with soluble and insoluble silicates, and finally found that the fine amorphous powder of non-purified silica—SiO₂—silicon dioxide (often labeled silicic acid) when used in a 5 per cent suspension in saline is as effective as the sol, is more uniform in action, is easily prepared, and not difficult to inject. In experiments with guinea pigs it produced much local tissue proliferation, numerous small foci of necrosis, and a marked proliferation of the injected tubercle bacilli. With white mice this silica also proved unsatisfactory.

At that time, a specimen of about 8 cc. of pleural fluid was brought to the laboratory for examination. The patient, a 17 year old girl, had become acutely ill a few days previously. According to her statement, she had never coughed before. The fluid showed a heavy pellicle. A smear showed no bacteria. The sediment obtained after centrifugation was digested with antiformon, neutralized, and a portion examined for acid fast bacilli with negative results. The rest I injected into a guinea pig which had first been given subcutaneously 1 cc. of the amorphous silica suspension.

The usual initial swelling almost disappeared by the third day, but became again quite palpable on the sixth day, and on the ninth day after injection there was present a firm infiltration about a centimeter in diameter. Believing that an abscess had formed, I decided to aspirate the pus, so that tuberculosis might still develop in case there were tubercle bacilli in the material injected.

I inserted a sharp stout needle on a 10 cc. Luer syringe into the center of the swelling and pulled the plunger. A strong vacuum formed in the barrel, but no pus appeared. I withdrew the needle and found it blocked by whitish tissue. A new needle gave the same results; it became plugged by firm tissue, apparently cut by the sharp edges of the needle and then aspirated into it. The tissue fragments thus obtained were spread into a thin smear on a slide and stained with Ziehl Neelson stain.

The microscope revealed the following: The tissue consisted of numerous young fibroblasts intermingled with epithelioid-like cells and various leucocytes, mostly mononuclear. Between these cells were seen many very brightly stained acid-fast bacilli, singly and in groups. The single bacilli often resembled incompletely developed chains of strepto-bacilli; those in groups were slender filaments, often parallel and bundle-like. Many of the bacilli were intracellular, in large vacuolated cells, the bacilli lying in the vacuoles.

I covered the puncture points with collodion, and kept the guinea pig alive for 4 more weeks. No local complications occurred, and on post mortem gen-

eralized tuberculosis was found. Cultures from the spleen grew very well on Corper's potato medium.

Realizing, of course, how extraordinary an occurrence, a positive inoculation diagnosis of tuberculosis in 9 days was, I performed a series of similar tests to check on my findings. For tuberculous material I used an emulsion of the spleen and liver of the tuberculous guinea pig. It was filtered through a double layer of filter-paper to remove clumps of bacilli, and diluted so that 0.5 cc. contained very few bacilli, as determined by smear. Guinea pigs of less than 300 grams in weight were injected subcutaneously with 1 cc. of the silica suspension, followed in 2-3 minutes with 0.5 cc. of the emulsion. Nine to ten days after injection there were firm infiltrated areas about a centimeter in diameter at the site of injection. When on the 10th or 12th day, specimens of tissue were removed from the infiltrated areas (by means of sharp stout needles well fitted to a 10 cc. Luer syringe), microscopic examination showed numerous acid fast bacilli, single and in groups, some intracellular, scattered between proliferating tissue cells. The bacilli stained very bright red, and were definitely in a state of active proliferation, as there were many times the number injected. No such results were obtained in the controls. Another interesting observation made in this series was, that when killed between 15 and 20 days after injection the "silicated" animals showed delayed dissemination of tuberculosis as compared with the controls, but in those killed at the end of 5 weeks the visceral involvement was much more pronounced than in the controls.

After numerous trials and long usage as a diagnostic procedure in the laboratory, I found that when the following rules were strictly observed my method yielded uniform and satisfactory results.

THE METHOD

1. Use only the fine amorphous non-purified silica powder (SiO₂). Place 1 gram of this silica into a sterile flask and pour over it 20 cc. of boiling hot physiological saline. Shake well and allow to cool before using.

2. In concentrating the suspected material care must be taken to cause minimum injury to the tubercle bacilli that might be present. Also, avoid as much as possible contaminating this material. After neutralization place it in the refrigerator until ready to inject.

3. Inject 1-1.5 cc. of the silica suspension subcutaneously into the flank of plucked guinea pig of less than 300 grams in weight. Use a sharp 17 guage stainless steel needle (Vim) on a 5 cc. Luer syringe. Keep the powder well distributed in suspension in the syringe.

4. Inject the concentrated material under investigation 2-3 minutes after the silica. Use a small needle, and insert it through the skin puncture made by the first needle, and to the same depth (about 1 cm.) and in the same direction as the first. In this way the material will spread over the area covered by the silica.

5. Cover the skin puncture with collodion, and place the animal in a cage. One small carrot daily will furnish all the food and drink it needs.

6. Examine the injected area every few days. If by the 10th or 12th day a firm infiltration (about a centimeter in diameter) has developed, obtain some of the exudate for examination.

For this operation use a sharp stout short needle (Vim) fitted well to a 10 cc. Luer syringe. Insert the needle so, that it traverses most of the infiltrated area, moving it slowly in various directions, and pull the plunger. Expel the material obtained upon a slide, spread it into a thin film and stain for acid fast bacilli.

If the first examination is negative, the same procedure should be repeated 2-3 days later.

The animal should be kept alive for four more weeks, as a check on the early findings.

Though the malignant influence of silicosis upon pulmonary tuberculosis had been recognized clinically for nearly a century, the nature of the interrelation between silica and the tubercle bacilli had not, as yet, been determined. Statistical studies have abundantly confirmed the high incidence of, and the appalling mortality rate from pulmonary tuberculosis among workers, who had been exposed for several years to silica dusts.

Experimentally, the most elaborate work on this subject had been done by L. U. Gardner and his co-workers in his series of

"dusting" experiments^{8, 9}.

The specific effect of silica upon the virulence of tubercle bacilli was interestingly demonstrated by L. U. Gardner in his experiments with the Saranac laboratory attenuated strain of tubercle bacilli (R I)^{10,11}. By "dusting" guinea pigs with silica containing dusts, Gardner succeeded in reactivating the self-limited and readily healing tubercles, usually produced by the R I strain, into a chronic progressive tuberculosis. When infection with R I was superimposed upon an already established silicosis, there developed an acute and rapidly fatal tuberculosis.

To my mind, a real clue to the nature of interrelationship between silica and tuberculosis was furnished by a recent statement of Gardner's¹², that the lesions of silicosis and tuberculosis are essentially similar, and that silica can cause every type of cellular response found in tuberculosis. Arguing from the above, if the cellular response—the reaction to injury—is similar, the injurious agents, the toxic action of the slowly dissolving silica and that of the bacilli and their products, are in some way (bio-

chemically?) similar. We know that the growth power of tubercle bacilli in the guinea pig following subcutaneous injection is determined by two factors (dosage being the same), the virulence of the strain and the time element. Silica, by enhancing the virulence factor, spares the bacilli much of the initial struggle against the defenses of the guinea pig, thus permitting them to proliferate much earlier. In my method of inoculation silica effects the results in yet another way. The rapidly proliferating fibroblasts and endothelial cells block the lymphatics and thus delay the dissemination of the tubercle bacilli. In this way a condition is created for early and abundant multiplication of the tubercle bacilli and their confinement to a small area.

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ENDOMETRIOSIS*

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The presence of endometrium in abnormal positions has been recognized for many years. According to Graves¹ the first knowledge of endometriosis dates back to 1860 when von Rokitansky described the adenomyoma of the uterus as a clinical entity. Cullen² is credited with describing the first case of endometrium located outside the uterus when he reported in the Johns Hopkins Hospital Bulletin in 1896 a case of adenomyoma of the round ligament. The first case of ectopic endometrium exclusive of adenomyoma in the English literature was that reported by Russell³ in 1899 when he described the presence of endometrium in an ovary. However, it was not until several years later, notably in 1919 and 1920, that the recognition of endometriosis exclusive of adenomyomata of the uterus became a common occurrence. In 1919 Casler4 reported the occurrence of menstruation in an endometrial cyst of an ovary. It was in 1921 that Sampson⁵ began his remarkable series of publications on the subject of endometriosis, and it is due largely to the intensive research of Sampson together with the theory of retrograde menstruction which he evolved, that the term endometriosis became familiar in medicine.

The etiology of endometriosis cannot be satisfactorily explained by any one theory, though undoubtedly the theory of retrograde menstruation with implantation of desquamated endometrium on peritoneal surfaces has more adherents than any other. However, there is one type of endometriosis which is fairly well understood, and that type is the uterine adeno-

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myomata. Cullen⁶ in 1908 was able to show that in 50 cases of adenomyoma of the uterus there was a direct continuation of the epithelium of the uterine mucosa with the epithelium of the endometrium in the adenomyomata. The uterine mucosa is normally invasive to a slight extent, and in the case of adenomyomata the invasive quality of the endometrium has been accentuated. The reason for the exaggerated invasiveness of the endometrium is unknown, but is probably related to the stimulating action of ovarian hormones on endometrial growth. We have often noted the unusually hyperplastic endometrium in cases of adenomyomata of the uterus.

In those cases of endometriosis which are not uterine adenomyoma the explanation of their occurrence is difficult. These cases of aberrant endometrium are explained chiefly by two theories, namely the theory of transtubal implantation, which was developed by Sampson, and the theory of coelomic heteroplasia, which was developed by Iwanoff, and the chief exponent of which is Emil Novak. Other theories such as those which explain the ectopic endometrium on the basis of blood and lymph stream metastases, and those based upon the assumption that they arise from fetal rests of Mullerian and Wolffian structures have received little attention.

Sampson believes that menstrual blood can and does sometimes regurgitate into the peritoneal cavity at time of menstruation, and furthermore that the desquamated endometrium may remain viable and implant itself on the peritoneal surface adjacent to the fimbriated end of the fallopian tube, or be carried elsewhere and implanted in the peritoneal cavity. The fact that the fallopian tubes are patent in endometriosis, and that desquamated endometrium has been found in the tubes of menstruating women lends support to the theory. Menstrual blood can be expressed from the fimbriated ends of the tubes of many menstruating women, and implants in the pelvis are in locations suggesting origin from the fimbriated ends of the tubes. The implants once occurring in the pelvis are known to seed themselves widely throughout the pelvic peritoneum. Sampson has shown that some cases of endometriosis are due to the implantation of tubal

mucosa in the ovary, and has traced a direct continuation of the epithelium of the tube with the epithelium of the ovarian implant. Jacobson⁸ was able by experimentation with rabbits to produce autotransplantation of endometrium, and Gay⁸ has been able to grow endometrium from menstrual blood, thus showing that desquamated endometrium contains viable epithelium and stroma cells.

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The major objection to the theory of transtubal implantation is that it cannot adequately explain the presence of endometrium in unusual situations such as the groin or in the umbilicus. theory of coelomic heteroplasia explains endometriosis on the basis of metaplasia of serosal cells of the peritoneum into epithelial cells, and the metaplasia of fibrous tissue of the peritoneum into stroma cells of endometrial type. The mucosa of the uterus, tubes, and vagina as well as the peritoneum itself develop from the coelomic epithelium, and it is maintained that the germinal epithelium of the ovaries or serosal cells elsewhere retain their potentiality of developing into Mullerian mucosa. The stimulus for this metaplasia is probably hormonal, or some unknown stimulus emanating from the fimbriated ends of the fallopian The coelomic heteroplasia theory easily explains those cases of endometriosis which occur in unusual situations as they rarely occur outside the various extensions of the peritoneal cavity.

The symptoms of endometriosis are variable and simulate many other pelvic lesions. The outstanding symptom is pain which appears in the form of dysmenorrhea or backache. The dysmenorrhea is of the acquired type and occurs ordinarily in women who have reached the age of endometriosis which is ordinarily from 30 to 50 years. The symptoms of endometriosis depend upon adhesions and the limitation of normal movement of pelvis viscera. Rectal pain is common due to involvement of the rectovaginal septum. Many cases simulate acute pelvic inflammatory disease, and in the absence of a history of pelvic inflammatory disease a cystic tumor of the ovary which is extremely painful should lead one to suspect endometriosis. Rupture of an endometrial cyst gives rise to symptoms not unlike those of acute

appendicitis. Implants can sometimes be felt or seen in the rectovaginal septum. Partial intestinal obstruction due to involvment of the sigmoid occasionally occurs, and implants in the bladder give rise to urinary symptoms. However, a large number of cases of endometriosis are "silent" in that they give rise to no symptoms.

Endometriosis is not a rare disease but is found frequently by those physicians who are aware of its incidence. Sampson found 159 cases of endometriosis in 664 operations for pelvic disease in women, or in other words in about 24 per cent. Hill, in reviewing the cases at the Touro infirmary found 135 cases of endometriosis in 1100 operations for female pelvic disease, or in about 12 per cent of the cases. Perhaps the major reason for the failure of a surgeon to find endometriosis at operation is the size of the implants. The majority of implants are about 2 mm. in diameter, and only careful observation will reveal their presence.

Since June 1933 there have been 89 cases of endometriosis at the Methodist Hospital in Memphis, Tennessee. In many of these cases there were multiple implants, particularly in the serosal type of involvement. The following list is an explanation of the sites of involvement and the types of involvement found in our cases.

		lumber of cases			umber cases
1.	Uterus	59	4.	Fallopian tubes	 5
	Adenomyoma	46	5.	Sigmoid	 5
	Diffuse type	19	6.	Bladder	 4
	Nodular type	27	7.	Abdominal scar	 3
	Serosa of uterus	15	8.	Omentum	 3
	Nodular adenomyoma and		9.	Small intestines	 2
	serosal involvement	10	10.	Cul de sac	 2
2.	Ovaries	42	11.	Broad ligament	 1
	Bilateral	22	12.	Uterosacral ligament	 1
	Unilateral	20	13.	Vesicovaginal fold	 1
3.	Rectovaginal septum	6			

The ages of these patients varied from 18 to 57 years, but the fourth decade contained 41 cases or slightly less than one half of the total number of 89 cases. A list of the occurrence of endometriosis according to decades in this group is as follows: second decade, 1 case; third decade, 8 cases; fourth decade, 41 cases; fifth decade, 28 cases; and 11 cases in the sixth decade. All of the cases occur-

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ses; curring in the sixth decade were of adenomyomata except one. All cases of endometriosis which occurred under 30 years of age were of the non-adenomyomatous type. The average age of the group as a whole was 38.9 years, the average age of the adenomyomatous group was 42.3 years, and the average age of the non-adenomyomatous group was 35.6 years. Thus the non-adenomyomatous group was on the average 6.7 years younger than the adenomyomatous group at the time of operation.

The preoperative diagnosis of endometriosis was made in 14 of the 89 cases. All cases except one which were diagnosed preoperatively were non-adenomyomatous. At the time of operation 33 cases of endometriosis were recognized as such by the surgeon, and 32 of these cases were the peritoneal type of endometriosis. Thus 58 cases of adenomyoma were not diagnosed at operation and 23 cases of serosal involvement were undiagnosed, showing a much greater tendency for the surgeon to recognize endometriosis when it involves the peritoneum than when it causes adenomyomata. It was found that adenomyomata are frequently mistaken for fibromyomata or fibrosis uteri. It must be admitted that several cases of the peritoneal type of endometriosis were discovered more or less accidently in routine pathological sections.

An attempt was made to determine if endometriosis was the cause of the operation performed, and in 69 of the total of 89 cases endometriosis was considered to be the primary cause of operation. Thus 20 cases were not the primary cause of operation and were "silent" in so far as being the cause of marked symptoms were concerned. Ten of these so called "silent" cases were of adenomyomata and ten were of peritoneal type of involvement.

An attempt was made to determine whether or not the uterine mucosa was hyperplastic in cases of endometriosis, and a rather large per cent was found to be associated with hyperplastic endometrium. Hysterectomies were done in all the cases where adenomyomata were found, and 37 of the 46 cases of adenomyomata were considered to be associated with an unusually thick endometrium. The endometrium was very translucent and juicy and the glands very tall and tortuous. The endometrium was often thrown into folds, and endometrial polyps often resulted from the extreme hyperplasia. The glands did not exhibit a "Swiss cheese pattern" as might be expected from an excess of folliculin alone. Endometrium was available in only 24 of the 43 c ses in which adenomyomata were not found. Fifteen of these cases were considered to have hyperplastic endometrium. Unfortunately the stage of the menstrual cycle was not available in all case histories. Witherspoon found endometrial hyperplasia in 64 per cent of 44 patients with endometriosis.

An interesting feature of the 27 cases of nodular adenomyomata was the presence of peritoneal implants in ten cases. This feature was not found in any of the cases of diffuse adenomyomata. The explanation of this phenomenon appears to lie in the fact that nodular adenomyoma often reach the peritoneal surface, and that at times of menstruation some of the superficial areas of endometrium in the adenomyoma rupture through the serosa of the uterus and

seed themselves in the pelvis. Sections of the diffuse adenomyomata failed to reveal any case in which the endometrial invasion reached within several millimeters of the surface.

The size of the implants varied markedly. The largest implant was a cyst 15 cm. in diameter. There were only 8 cases in which endometrial cysts reached 5 cm. in diameter. Of interest also was the fact that two cases had partial intestinal obstruction from endometriosis of the sigmoid. Intestinal resection was necessary in one case. Two cases came to operation because of rupture of endometrial cysts. Of the three cases which occurred in abdominal scars one followed caeserian section, one followed salpingectomy, and one followed hysterectomy.

The pathological picture of endometriosis depends upon the demonstration of endometrium in abnormal positions. endometrium must be composed not only of the endometrial glands, but also the stroma cells of the endometrium. In the stroma are also found the small round cells which resemble lymphocytes. The endometrium performs the function of menstruction in the abnormal position, and following removal of the ovaries or sterilization by x-ray the function of menstruation ceases. In the adenomyomata of the uterus there is a simple extension of the endometrium from the surface of the uterine mucosa into the myometrium. Endometrial cysts in the myometrium do not reach a large size due either to the pressure of the surrounding muscle or due to a drainage of the glands into the uterine cavity. Occasionally there is a slight retention of menstrual blood in the endometrium of adenomyomata, and menstrual blood is found phagocytized by large mononuclear cells. A scar tissue growth in the surrounding smooth muscle is also probably due to the irritation of retained menstrual blood.

Where endometrial implants appear outside adenomyomata the growth originates as small purple nodules in the surface of the peritoneum. These nodules at first are about 2 mm. in diameter, and when punctured by the knife exude a small quantity of chocolate colored material. Surrounding the early implants are delicate cobweb like adhesions, which when removed reveal a puckering of the peritoneal surface in a radial manner for the distance of about 1 cm. from the implant. As the implant grows older, and the patient passes through one or more menstrual

cycles four things will usually take place. First, the cyst is prone to rupture and seed itself further in the peritoneal cavity, second, it will adhere to adjacent organs and implant itself into that organ by extension, third, it will invade further into the viscus in which it lies, and fourth, the irritation of the retained menstrual blood will arouse a marked inflammatory reaction in the surrounding tissue with the formation of very dense scar tissue. The adhesions produced by a long standing case of endometriosis are said to be much more firm than those of pelvic inflammatory disease.

The demonstration of endometrium is a simple matter in the small implants and the pathologist will be wise to select these areas when taking his specimens. The small implants are too early to be distorted by the retention of menstrual blood. As the implants become older the presence of menstrual blood overshadows the presence of endometrium, and it is quite difficult to demonstrate endometrium in the walls of the larger cysts. The large cysts are found to be lined by a granulation tissue in which phagocytized blood is very prominent. There is an infiltration of this granulation tissue with large mononuclear cells, with an occasional polymorphonuclear neutrophil, and with a few lymphocytes. At times foreign body giant cells are observed attempting to remove the retained menses. The thickness of the wall of the cyst depends upon the age of the implant. If endometrium is found lining the large cyst it will frequently be atypical, being often composed of a single layer of columnar cells without glands or stroma. However, multiple sections through the wall of the large cyst will show endometrium of the characteristic type, and this endometrium will most likely be found in the scar tissue at the periphery of the cyst.

In conclusion we think it not amiss to mention those conditions which are often mistaken for endometriosis of the ovary. Cysts of the ovary which contain a viscid chocolate colored fluid are occasionally endometrial implants, but very often a cyst of a corpus luteum, or hemorrhage into a follicular cyst produce a similar picture. Small cystadenomata into which there has been much hemorrhage are also often mistaken for endometriosis.

CONCLUSIONS

1. Eighty-nine cases of endometriosis are reported. Forty-six cases were of adenomyoma, and 53 cases, were of endometriosis other than adenomyoma. Ten cases showed both adenomyoma and peritoneal involvment.

2. Endometriosis is a disease chiefly of the fourth and fifth decades.

3. Hyperplasia of the uterine mucosa accompanies a large percentage of cases of endometriosis, particularly in adenomyoma.

4. The hyperplastic endometrium in endometriosis differs from the usual picture found in excess secretion of folliculin.

5. The nodular type of adenomyoma differs from the diffuse type in that the nodular type is frequently complicated by peritoneal endometriosis due to extension from the adenomyoma.

6. The demonstration of the endometrial character of large endometrial cysts is difficult.

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FOREIGN BODY IN HEPATIC DUCTS PRODUCING OBSTRUCTIVE JAUNDICE OF SEVEN YEARS' DURATION WITH INTERNAL BILIARY FISTULA*

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Foreign bodies in the biliary passages may gain entrance from the stomach or intestines or at the time of surgical procedures on the biliary tract. The suspected presence of a lesion of this type rarely enters into the differential diagnosis of biliary colic or obstructive jaundice because of its rarity and lack of specific symptoms. A diversified group of objects have been found under these circumstances, such as swabs, thread, bristles, gauze, fruit seeds, cherry stones, steel needles, pieces of wire, handle of spoon, bullets, rubber drains, and absorbable and non-absorbable suture material (Toland¹ and Rollestone and McNee)². One of the unusual features in certain cases of biliary tract foreign body is the long duration of the symptoms as illustrated in the case here presented and in a number of the cases reviewed in the literature.

REPORT OF A CASE

This case was presented before the Philadelphia Pathological Society, November 12, 1936.

M. S. White female, single, aged 33 years, was admitted to the Jefferson Hospital 5–11–34 with marked jaundice. Attacks of jaundice had been recurrent at intervals over a period of 7 years following cholecystectomy and appendectomy. The cholecystectomy had been performed in 1927 for "bilious attacks" associated with nausea, vomiting and severe epigastric pain of one year's duration. Very little could be learned about the details of this operation as it had been performed at a distant hospital. Following inquiry the diagnosis was given as chronic appendicitis and cholecystitis, with appendectomy and cholecystectomy, the condition of the patient on discharge being described as

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good. According to the patient's story there was evidently considerable trouble following cholecystectomy. Drainage was continued for seven weeks and a second operation was performed, presumably appendectomy, followed by drainage for four weeks. The entire period of hospitalization was four months.

Only three weeks after leaving the hospital, in 1927, she developed painless jaundice accompanied by pruritis, dark urine and clay-colored stools. These symptoms persisted for two or three weeks and then subsided, recurring subse-

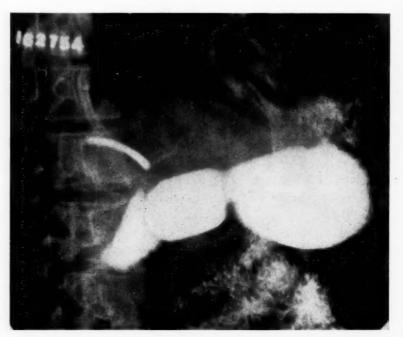


Fig. 1. X-ray Film of Abdomen Following Ingestion of Barium The long metallic foreign body is present in the right upper quadrant.

quently at fairly regular intervals of about 2 months. During the past three years, the patient bled frequently from the nose and from practically any traumatic lesion however slight. Although some degree of clinical icterus was usually present, even in the intervals between the major attacks, there was no history of abdominal pain at any time since operation. Jaundice became pronounced 6 months prior to the present admission and persisted until death.

Physical examination revealed marked jaundice. The spleen and liver were both palpable below the costal margin on inspiration. There was some tenderness over the upper border of the right rectus muscle but definite rigidity could not be elicited. Small placques of xanthelasma were observed on the left upper eyelid and along the lateral margins of the nose. Roentgenographic study of the right upper abdominal quadrant 5–16–34 disclosed the presence of a long, thin, dense, metallic body, curved slightly to the right and directed anteroposteriorly, the longest measurement of which was estimated at approximately 3.3 cm. (fig. 1). There was a marked degree of secondary anemia. The blood platelets ranged between 254,000 and 346,000. The urine contained a trace of albumen and hyaline and granular casts. The Van den Bergh reaction was positive direct; the serum bilirubin varied between 5.12 mgm. and 10.10 mgm. per 100 cc. On different occasions, the bromsulphalein retention varied between 40 and 100 per cent at the end of 30 minute periods (2 mgm. dosage). The blood cholesterol was 198.

Therapeutic biliary drainage was employed and bile obtained in the drainage in each instance. Other treatment was directed to combat the severe anemia and improve the hepatic function to permit surgical measures for the relief of obstructive jaundice. The patient seemed to improve for a time, but five weeks after admission she began to regress rapidly. There was profuse epistaxis, the blood platelets dropped to 140,000 and the blood coagulation time was 15 minutes and the bleeding time 12 minutes. She vomited large amounts of blood and developed extensive areas of subcutaneous hemorrhage the day preceding death, which occurred 7–5–34.

Post-mortem examination

The autopsy, which was limited to examination of the abdominal contents, was performed 7½ hours post-mortem. The mucous membranes and skin were pale and jaundiced. There was an old healed scar in the right upper, and another one in the lower right quadrant of the abdomen. The peritoneal cavity contained a large amount of dark, bloody fluid and the serosa was everywhere congested. The esophagus, stomach and proximal part of the small intestine were greatly distended with dark, greenish fluid and gas. The pyloric end of the stomach was displaced and attached by many adhesions to the under surface of the liver at the site of the old fossa of the gall bladder. The duodenum was markedly dilated and, just below the pyloric ring, contained a fistulous opening from which bile could be expressed. The mucosa of the ileum was congested in areas and for a distance of 40 cm.

In the midportion the entire circumference of the wall and corresponding mesentery was hemorrhagic. Below this point, the remainder of the ileum and the colon contained a large amount of bloody fluid.

The liver weighed 2600 grams and measured 27 x 24 x 10 cm. (fig. 2). It was tough, finely granular, irregular in shape and mottled dark green. The right lobe which was large and dome-shaped, was surrounded by many adhesions. On section, a large amount of pale, ropy, light, greenish fluid escaped from the greatly dilated intrahepatic biliary passages. The left hepatic duct averaged 3.5 cm. in diameter practically throughout its entire course. The usual mark-

ings of the liver substance were replaced by small, pale, yellowish green areas, measuring less than 1 mm. in diameter, which stood out against the dark green of the surrounding tissue. The gall bladder was absent.

The common bile duct, which was patulous throughout and somewhat dilated, formed an acute angle near its origin and ran an extended course of 12 cm. emptying into the duodenum at the usual location. The duodenal fistulous



Fig. 2. Liver

The surface of the liver is covered with adhesions. The common bile duct (A) terminates in the duodenum at (B). A white probe is inserted into the fistula. To the right of the pole, the foreign body may be seen in the bile duct.

tract, mentioned above, admitted a probe of 1 mm. in diameter and opened into the common hepatic duct at what appeared to be the junction point with the common bile duct. The common hepatic and the right hepatic ducts were markedly dilated, the latter measuring 1.5 cm. in diameter. The foreign body was found to be a blade of a hemostat, which measured 4.2 cm. in length, and which occupied the lumen of the common hepatic and first portion of right hepatic ducts (fig. 3). The foreign body was coated over with dark green, friable, pigment concretion. The sharp fractured end of the blade pointed in the direction of the duodenal fistulous opening. The left hepatic duct was considerably dilated above its orifice.

Microscopic description of liver

The hepatic parenchyma was divided into small nodules by bands and dense masses of pigmented connective tissue containing necrotic hepatic cells, a few proliferating bile ducts and many foci of inflammatory cells chiefly lymphocytes.



Fig. 3. Curved Blade of Hemostat Found in Right and Common Hepatic Ducts

The portion on the left was covered by the friable calculus lying below. The sharp fractured edge on the right pointed in the direction of the duodenal fistulous opening.

There was considerable distortion of the architectural pattern; the hepatic cells were swollen, the cytoplasm granular, and the nuclei poorly stained, and many contained bile pigment. The large intrahepatic branches of the bile ducts were dilated, the epithelial lining cells were necrotic and many had desquamated and the walls of the ducts were thickened and fibrous.

COMMENT

Foreign bodies in the gall bladder are not uncommonly met with usually the result of material such as sutures, sponges, etc. left behind after operation, but foreign bodies found in the bile ducts are very rare except as pathological curiosities. nosis of the presence of a foreign body in the biliary passages was made preoperatively in this case by means of the roentgenological Because of the patient's critical physical condition examination. and lack of improvement, surgical intervention was out of the question. It was obvious at this point that the patient was suffering from hepatic insufficiency, the result of the long standing biliary obstruction. It has been pointed out by others that a foreign body may gain entrance to the biliary tract by migration from the gastrointestinal tract or peritoneal cavity or directly by accidently leaving them in the ducts after operation. However, since in this instance no detailed description of the previous operation could be obtained, it is impossible to throw any light on the mode of entrance of the foreign body into the biliary passages. If the nature of the obstruction had been recognized earlier before the damage to the liver was so great, it could no doubt readily have been corrected by surgical intervention. The periodical attacks of jaundice and the clinical manifestations during the last 7 years of the patient's life, were similar to those produced by stone in the common bile duct. The complete absence of pain in these attacks, however, is a notable feature. body was situated within the right and common hepatic duets, in which the formation of gall stones is rarely demonstrable. It acted as a nucleus for the incrustation of bile salts and pigment which were deposited irregularly on the surface increasing the thickness of the object several times. Whether or not the amount of calculous deposit on the foreign body played any part in the symptoms of obstruction is difficult to say but was suggestive. It is interesting that the wire in Cooke's case and the drainage tube in Federoff's4 case had undergone no appreciable change although the latter object had been resident in the common bile duct for $5\frac{1}{2}$ years. Calculous formation has been observed in

association with suture material in the biliary passages in several instances, with cotton in the common bile duct (Toland) and with a gauze sponge which remained in the gall bladder for 11 years (Bevan)⁵.

The effects on the liver of the recurring attacks of biliary obstruction resulted in chronic hepatitis, with pigmentation, marked fibrosis and destruction of the lobular pattern to an extent rarely observed, except in the liver of portal cirrhosis. As with stone in the common bile duct, the intermittance and degree of obstruction, the number and duration of the obstructive periods, the length of sojourn of the foreign body in the bile ducts, coupled with the presence of infection, combined to produce hepatic lesions of a marked grade of severity. The development of the duodenal fistula in this case did not obviate the effects of biliary stasis, since the communication was established at a point below that at which impaction in the bile ducts were occurring. Aside from alleviating the effects of any obstruction which might have resulted from the marked angulation of the common bile duct, the only possible consequence of the fistulous tract was to permit regurgitation of duodenal contents into the bile ducts. Although no evidence was obtained roentgenographically that this occurred to any extent following ingestion of barium, it is a logical assumption in view of the dilatation and marked obstruction of the duodenum by adhesions, which were demonstrated postmortem.

CONCLUSIONS

A case is reported of obstructive jaundice of 7 years duration due to a calculous encrusted foreign body (blade of hemostat) in the hepatic ducts, with a duodenal fistulous communication. The manner in which the foreign body gained access to the biliary passages could not be determined.

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PRIMARY CARCINOMA OF THE LIVER IN A CHILD*

CASE REPORT

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Although primary carcinoma of the liver is a relatively rare disease, yet a considerable number of cases have been reported in children and since malignancy in children is uncommon, this type of carcinoma represents a relatively higher incidence than among adults. This case represents an interesting clinical study whose signs and symptoms did not point to liver pathology, so this diagnosis was not considered.

Steiner¹ has recently reported two cases of primary carcinoma of the liver in children (a 4 month old girl and in a 16 month old boy) and reviewed the literature completely and summarized the cases reported. In addition to these cases, he found 75 cases of authentic carcinoma of the liver in children. It is quite interesting that 53.2 per cent of these occurred in children under the age of two years. Of the cases reported, 68 per cent were in boys and 32 per cent were in girls. The symptomatology was variable, and the average duration of life was 4 months. Of these cases, 52 were hepatomas, 3 were cholangiomas and 22 unclassified. There were metastases in 27.2 per cent of the cases and many of these were to the lung. To this series there should be added a case reported by Hamberger². This, together with the case reported here, makes a total of 79 cases.

CLINICAL SUMMARY

The patient, a well-developed white male child aged two, was admitted to the University Hospital in April 1938. The child had been in good health until two weeks before admission when he became irritable but not acutely ill. At this time the mother felt a lump below the rib margin on the right. He was

^{*} Received for publication June 3d, 1938.

treated with X-ray but showed little improvement and the parents were then advised to bring the child to the hospital. Physical examination was essentially negative except for a movable, firm, irregular mass lying beneath the right costal margin extending to within 2 cm. of the iliac crest. The mass did not move with respiration, and there was no jaundice or ascites. Urine examination showed 3 plus albumen, occasional red blood cells, and 25–50 white cells per high power field. A tentative diagnosis of neoplasm of the right kidney was made. X-ray taken on 4–20–38 showed the right kidney to be somewhat enlarged, and the appearance was considered compatible with a Wilm's tumor. Retrograde pyelograms showed a medial displacement of the ureter of the right

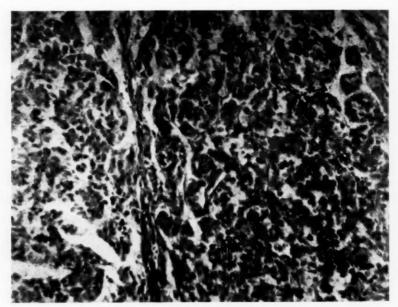


FIG. 1. PRIMARY TUMOR

kidney, consistent with a retro-peritoneal tumor arising in the region of the lower pole of the kidney. The therapy report given by the radiology department on 6–9–38, was as follows; "The tumor of the right kidney had been previously treated from 4–5–38 to 4–13–38 with 1000 R. anteriorly and 750 R. posteriorly. This treatment was carried out before the patient's admission here. From 4–30–38 to 6–4–38, 2,938 R. were delivered posteriorly and from 4–30–38 to 5–31–38, 2,145 R. and 1,670 R. laterally. The tumor was reduced to one-third its original size. The patient was discharged on 6–5–38."

The patient was readmitted on 7-7-38 for a check-up examination. Since his previous admission he had been in fairly good health and his mother stated

that the lump had disappeared. On physical examination no mass was palpable It was felt that irradiation had produced such a diminution in the size of the tumor that it would be amenable to surgery. On 7-11-38 a right nephrectomy was done. Exploration of the region revealed no gross evidence of tumor, however, it was felt that some tumor remnants might be present and a nephrectomy was therefore done. Grossly the kidney was entirely normal in appearance, and microscopically it showed no inflammatory or neoplastic involvement. The child pursued a quiet post-operative course and was dismissed on 7-20-38.

The child was re-admitted to the hospital on 9-6-38. At this time he had been restless and suffered from anorexia for a week. For three days before

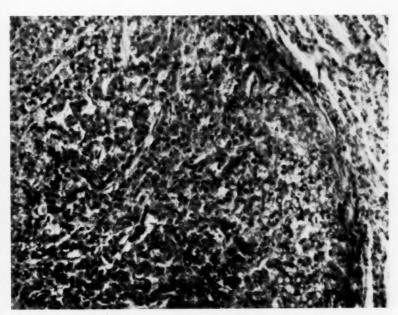


Fig. 2. Lung Metastasis

admission, he had had pain in the right side and flank, and had vomited two or three times daily. Upon admission the rectal temperature was 104, pulse 145 and respirations 42 and examination revealed a considerable mass or liver enlargement in the right upper quadrant. The abdomen was distended with gas. The spleen was not palpable. The white count was 25,200. X-ray examination on 9–9–38 showed round areas of increased density in both lungs suggesting metastatic neoplastic nodules or infarcts of the lung. No jaundice was present. The abdomen was distended and tympanitic. It was felt that the high white count was due to an abscess at the site of the old nephrectomy from which there was still a small amount of purulent drainage. The wound

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was explored but no definite abscess cavity was encountered. The child became progressively worse and died on 9-10-38.

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Autopsy findings

The peritoneal cavity was filled with cloudy yellow fluid and the loops of bowel were covered with purulent exudate. The peritonitis was found to have extended from the infection at the site of the nephrectomy. The right lumbar region showed the presence of granulation tissue and purulent exudate in the bed of the kidney. Gross serial sections showed no evidence of neoplasm. The liver weighed 540 grams. Poppoletta and Wolbach³ give the normal liver weight as 394 grams for a child aged two. There was a neoplastic nodule about 1.5 cm. in size extending from the anterior inferior border of the right lobe of the liver. The right lobe showed the presence of numerous neoplastic nodules on the inferior surface. On cut section it was seen that this lobe was about half replaced with neoplastic tissue. The nodules were soft, light yellow in color and the larger ones showed central hemorrhage and degeneration. The gall bladder was not involved. The pancreas showed no neoplastic involvement. Some enlarged pre-aortic lymph nodes were seen but grossly and microscopically showed no evidence of neoplastic involvement. The adrenals were entirely normal. The prostate was not enlarged and there was no tumor palpable in the scrotum or inguinal canal.

The pleural cavities contained a small amount of clear yellow fluid. The left lung weighed 70 grams. There were numerous nodules 3–5 mm. in diameter beneath the plural surface. A few of these nodules were also present in the lung substance. The nodules were yellowish-green in color and the larger ones showed central hemorrhage and degeneration. After fixation the nodules were quite green in color. The right lung weighed 100 grams and was essentially similar to the left. Both lungs showed patchy consolidation.

Microscopically the liver showed large areas of neoplastic involvement. The neoplastic cells were relatively large, columnar in form and had a finely granular cytoplasm. The nuclei were central, light staining, rather large and round and the appearance of the neoplastic cells was extremely similar to that of normal liver cells. There were numerous small vessels present and the neoplastic cells tended to arrange themselves in cords about these vessels so that they simulated liver tissue architecture. In some regions the cells were so regular that they were distinguished with difficulty from the normal liver cells. There was no duct proliferation but some deposit of bile pigment was present. Portions of the neoplasm showed central necrosis and old hemorrhage. In some regions the neoplasm grew in masses separated from one another by dense connective tissue bands giving an appearance similar to that seen in cirrhosis. Many of the neoplastic nodules showed vacuolization of the cells which fat stain showed was due to deposition of lipoid material giving an appearance like that of fatty degeneration of the liver. In some regions the

fatty degeneration was zonal in distribution involving the intermediate zone of the neoplastic "lobule." Fat stains showed considerable lipoid deposit in the necrotic regions. Sections from the left lobe showed no neoplastic involvement but did show cloudy swelling and fatty degeneration.

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Sections taken from the neoplastic nodules in the lung showed an identical type of growth. Cord formation, bile production or retention and fatty degeneration was seen in the neoplastic cells here as was seen in the neoplasm in the liver. Some of the sections closely resembled normal liver tissue. Some of the alveoli near the neoplasm showed some round cell infiltration; inflammatory changes in the lung, however, were not striking.

Sections taken of the tissue at the site of the nephrectomy showed the presence of a non-specific granulation tissue, but no evidence of neoplasm.

The extreme similarity between the neoplastic cells and the normal liver cells was most striking. They were distinguishable by the absence of ducts and well-formed liver architecture and by slight irregularity of the neoplastic cells. Ehrich⁴ has shown that variations in the size of the nuclei of tissue cells are related to the degree of anaplasia. Since these neoplastic cells were so similar to those of normal liver it was felt that this variation would serve to make a quantitative distinction between them. The maximum, minimum, mean and standard deviation of the nuclear diameters of normal liver, liver tumor, and lung metastasis are listed below:

	MAXIMUM	MINIMUM	MEAN	STANDARD DEVIATION
Normal liver	7.7	4.9	6.08	0.54
Liver tumor	9.1	4.9	6.58	1.17
Lung tumor	9.1	3.5	5.88	1.20

It is seen that there is little difference between the mean values of the nuclear diameters for the normal cells and tumor cells. However, the standard deviation which measures the degree of irregularity of size of the cells is more than doubled in the tumor cells.

SUMMARY

- 1. A case of primary carcinoma (hepatoma type) in a child was reported, making a total of 79 cases.
- 2. The degree of variation of the nuclear diameters was computed and compared with that of normal liver.

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EDITORIAL

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THE TUBERCLE BACILLUS AND VIRULENCE

The clinical pathologist is frequently confronted with the problem of determining the virulence of a particular microörganism found in and producing certain pathologic changes in the human economy. In many cases, the subject is simplified when the microörganism is so pathogenic that only small numbers are needed to initiate similar pathogenic changes in a highly susceptible and suitable test animal. It is simplified also when the strain is a strict pathogen and is found only in the lesions of the disease transmitted from man to man. However, when the microörganism is found in nature (as are many of the mycotic fungi) and, in addition, can grow under certain conditions not restricted to body temperature, the complications become greater for implicating these microörganisms as the cause of the disease in an individual case. Thus, for instance, monilia, torula, and coccidioides imitis have been found at postmortem in the same individual.

Though not conclusively demonstrated in the case of a certain microörganism, sometimes the existence of ultramicroscopic forms has led to rather embarrassing explanations in the past, which prove amusing at times when viewed in the light of our present knowledge. The human tubercle bacillus has run an interesting gamut of maze-like attributes of virulence in an endeavor to explain results, which would have been simple had the analysis been made on the basis of an actual knowledge of virulence itself.

Thus originated the amusing statements not many decades ago, to mention only a few¹ that viability of a certain strain is lost while virulence remains, and that virulence is a rapidly changeable and fluctuating factor of the bacilli. These assumptions were derived from data resulting from the erroneous deduction that a certain medium would support the growth of few bacilli,

which it would not, and that virulence alone determined the fate of the animal, which in this case happened to be highly susceptible to the strain used. Today we know that so far as the tubercle bacillus is concerned, we can safely say of an individual bacillus: without viability, there can be no virulence. Today also, we fully realize that dead bacilli can produce tubercle when present in sufficient amount in one place in the human or animal economy² but that tubercle is not a criterion of virulence³.

The introduction of BCG (Bacillus Calmette-Guerin), an avirulent bovine tubercle bacillus, into practice by Calmette from 1921 to 19244 and the subsequent accidental Lübeck disaster introduced transient confusion but stimulated scientific activity to a point where the problem of the virulence of human and bovine tubercle bacilli had to be studied more accurately. This study has served certain important fundamental purposes which should be helpful in discussing virulence in general. Foremost among the disclosures, which should have been recognized ever since the discovery of the bacillus in 1882 but particularly since 1898 when the two pathogenic mammalian and the avian strains were biologically recognized, was the fact that the determination of virulence must be based strictly on quantitative evaluation⁵. Within the limits of practical approximation at least, we must be able to say that a certain number of bacilli will initiate disease in a normal animal (of standard proportions and not previously having contact with this or other microörganisms disturbing these normal relations so far as resistance is concerned). In the case of the mammalian (human and bovine) tubercle bacilli, the guinea pig proved of inestimable value for these studies because it is highly susceptible to infection with very small numbers of highly virulent, naturally occurring, human or bovine tubercle bacilli recently isolated from the disease in man or cattle.

The introduction of the newer accurately evaluated delicate culture tests for determining the presence of the number of viable bacilli also proved invaluable. With this means speculation was replaced by accurate test, and virulence could be more nearly evaluated. In the case of the human and bovine tubercle bacilli, another obstacle was removed in the interpretation of virulence

when it was recognized that very small lumps of bacilli could produce tubercle: that heavy suspensions of bacilli would inadvertently contain lumps sufficiently large to produce tubercles: and, if tests are to be made with crude heavy suspensions, the final word on virulence must be determined with suspensions fine enough to avoid initial tubercle formation from the original suspension, the focal accumulation at the site of injection, or in organs of deposit⁷ when given intravenously. From such careful evaluation, we can now say that human or bovine tubercle bacilli (shortly after isolation from human and bovine disease sources) may be so virulent (or pathogenic) that from 1 to 100 bacilli (1 billionth to 1 ten-millionth milligram) can infect a normal guinea pig and produce a progressive tuberculous disease; and after prolonged artificial cultivation, they may become so avirulent that 100 milligrams given intravenously will not produce a progressive disease in this susceptible animal.

The criterion of virulence is not tubercle formation, as assumed by some observers of several decades ago, but evidence of the multiplication of the bacilli in the susceptible host. On this basis, there now are in existence three well known strains of avirulent human and bovine tubercle bacilli of reasonable avirulent stability, based on suitable tests: Mucobacterium non-phymatiosis (bovine, variety BCG); mycobacterium non-phymatiosis (human, variety R 1, Trudeau); and mycobacterium non-phymatiosis (human, Corper)8. These bacilli may serve valuable practical purposes in that they are tubercle bacilli and not acid-fast saprophytes and their use may further elucidate the many intricate problems of tuberculosis by comparison tests with mycobacterium tuberculosum (much evidence for this contention has accumulated within this decade). This simple criterion of virulence for mammalian tubercle bacilli should also serve as a simple basic model for determining the virulence of other microorganisms on a quantitative basis, using numbers and pathogenicity as evidence. In passing, it might be added that as yet no crucial evidence exists that the mammalian tubercle bacilli fulfill exacting requirements for the existence of ultramicroscopic forms.

H. J. CORPER

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NEWS AND NOTICES

A Joint Committee of Specialties, comprising Anesthetists, Radiologists, Physical Therapists and Pathologists was recently formed in New York State with Dr. M. J. Fein as Chairman and Dr. Madge C. L. McGuinness as Secretary.

The March issue of the Mississippi Valley Medical Journal, is the "Twelfth Annual Radium Number" of this Journal and contains ten articles, mostly by well-known American radiologists, covering the recent advances in the field of radiation therapy.

RECOMMENDATIONS OF THE AMERICAN PUBLIC HEALTH ASSOCIATION TO THE TECHNICAL COMMITTEE ON MEDICAL CARE

1. It is certainly theoretically desirable that a single state agency should be made administratively responsible for carrying out all the provisions of the National Health Program which may be enacted into law.

In recommending that this single agency should be the state department of health, we recognize that the present patterns in most states do not conform to this proposal, yet we note evidence that organized medicine and many public welfare officials share our opinion that at least ultimately the state health department should be the responsible agency. We believe that there are many affirmative reasons why the state health department is the best agency at the state level for this purpose. No agency will be able so readily or effectively as the health department to provide professionally qualified personnel and be so readily or effectively able to maintain high professional standards of medical care.

In recommending that the state health department should be the primary integrating and coördinating unit, we recognize that the counsel of qualified advisers from the medical, dental, nursing, hospital and ancillary professions will be requisite, that adequate provisions for technical staffs and administrative expense will have to be made from the outset, and that increased funds for training purposes will be essential for successful performance. We have concluded further that, however, reluctant medical health officers may be at present to take over these added responsibilities, a study of the alternative choices for such purposes will be determinative. This basic recommendation does not preclude a working arrangement in some states with existing machinery outside of the official health department which might function well through another channel, provided that the state health officer retains supervisory control over the broad plans and the general purposes of the funds which the state may

receive. It is further recommended that in such plans due consideration will be given to the allocation of funds by a state department of health to the various substantial governmental jurisdictions within a state where population, extent of the special problems or financial need justify.

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We note that this proposal is in accord with the recommendation of the Interdepartmental Committee that this program should be developed around and be based upon existing preventive health services.

- 2. The Committee reaffirms and reemphasizes the official declaration of the American Public Health Association that, in the initiation and development of the program, wide latitude should be given to the states in the definition of the population to be served, in the selection of the method of providing medical service, and in other important phases of the proposed program. We believe that similar latitude should be provided with regard to the method of raising funds in the states to accomplish approved objectives.
- 3. The Committee finds itself in agreement with the recommendations in the National Health Program that the fundamental objectives involved here are, first, conservation of health and vitality and, second, reduction of the rôle of sickness as a cause of poverty and dependency. With this in mind, it supports the concept that Recommendations 1, 2 and 3 of the Interdepartmental Committee (the expansion of public health and maternal and child health services, the expansion of hospital, clinic and other institutional facilities, and the provision of medical care for the medically needy) should have priority in initiation.
- 4. We believe that recent experience demonstrates that the Social Security Act provisions for aid to the states for health work provide a suitable framework for the expansion of preventive health services.
- 5. We submit that it is essential that any state program to be approved for federal aid should contain adequate provisions for the maintenance of high personnel standards and that payment of such federal aid to state agencies should be withheld when it is found that substandard services are being furnished. Similar policy should obtain with respect to state aid to local areas within a state. The appropriate federal administrative authorities should have power to establish minimum standards through rule and regulation after consultation with competent advisory professional bodies.
- 6. Careful study will be necessary to perfect administrative regulations to cover the details concerned with the provision of medical services, so as to assure a high level of quality. We believe that standards of medical practice should not be written into basic law. Federal aid should be conditioned on

inclusion within the state plans of adequate safeguards for maintaining appropriate standards.

7. We believe that the extension and improvement of public health services in general throughout the country requires complete integration of health services of the federal government under one cabinet officer, preferably a Secretary of Health.

Notice of Temperature Symposium

A symposium on "Temperature and its Measurement in Science and Industry" will be held under the auspices of the American Institute of Physics, probably next fall, the dates to be announced later. Consistent with the title, the symposium will broadly cover many fields, its primary purposes according to present plans being to (1) coördinate the treatment of the subject in the sciences and branches of engineering, (2) review principles and bring up to date the record of recent work, (3) accumulate contributions for a comprehensive text, to be published as soon as possible after the symposium is held, (4) reveal the subject as an important branch of physics and (5) supply schools with the information required for the improvement of curricula. The Institute confidently expects that a stimulating, valuable and unified program will be arranged, an aim which will require the help of many contributors.

A representative steering committee has been formed consisting of the Chairman, C. O. Fairchild, Director of Research, C. J. Tagliabue Mfg. Co.; Dr. E. F. DuBois, Medical Director Russell Sage Institute of Pathology and Professor of Medicine Cornell University Medical College; Dr. Gustav Egloff, Director of Research, Universal Oil Products Co.; Dr. John Johnston, Director of Research, U. S. Steel Corporation; Dr. Walter G. Whitman, Head, Department of Chemical Engineering, Massachusetts Institute of Technology; and

Dr. H. A. Barton, Director, American Institute of Physics.

Those who are interested in taking part in this symposium should communicate with the Institute (American Institute of Physics, 175 Fifth Avenue, New York, N. Y.) at an early date, giving information regarding their field of work and the subject of the contribution they wish to make. Such contributions will be coördinated with the subjects of a group of invited papers, and assignments and divisions made. Further information for contributors will be available shortly.

BOOK REVIEWS

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The Pneumonias. By Hobart A. Reimann, M.D., Professor of Medicine, Jefferson Medical College, Philadelphia; Formerly Professor of Medicine, University of Minnesota and Associate Professor of Medicine, Peking Union Medical College, Peking, China. Cloth, 381 pp. 111 figures. \$5.50. W. B. Saunders Co., Philadelphia.

This is a particularly timely book. Few are better prepared to discuss pneumonia than Dr. Reimann for, by virtue of his special interest in the disease and an extensive experience as a clinician and teacher, he has acquired a comprehensive knowledge of the disease in all its phases and has made many important contributions to its study.

The book is divided into three main sections in which are discussed, The Specific Forms of Pneumonia; Pneumonia As A Specific Form Occurring As Part of a Systemic Disease; and Pneumonia Secondary To Acute and Chronic Diseases, Mechanical Causes, Shock, Senility, etc. Caused by Mixed Infections.

The book is comprehensive in outlook and thorough in presentation. The style is easy and readily understandable.

While it can never be said in medicine that the last word has been spoken, Dr. Reimann's book may well be said to be the last word on pneumonia to date.

His book can be unreservedly recommended to the physician, investigator, pathologist and student as a noteworthy contribution destined to be a standard reference text.

The American Illustrated Medical Dictionary. By W. A. Newland Dorland, A.M., M.D., F.A.C.S. With the collaboration of E. C. L. Miller, M.D. 18th Ed. Leather, 1607 pages, 942 illustrations. \$7.50. W. B. Saunders Co., Philadelphia.

A dictionary is a somewhat difficult book to review. For it is seldom read as a book (though it is surprising how interesting it can be to browse idly in one) and most often turned to for help. And, in view of the astonishing rapidity with which new terms appear in medical literature, a dictionary is almost essential as a vade mecum.

Happily, "Dorland" requires neither introduction nor commendation. For thirty two years it has been a well known and well recognized standard book. Suffice it to say, this new edition embodying over 3000 new terms, maintains the standard of excellence of its predecessors. The doctor, and the medical writer, especially, cannot afford to omit a new dictionary from his reference shelf.

The Cause And Prevention Of Disease. By WILLIAM HARVEY PERKINS, M.D., Professor and Director of The Department of Preventive Medicine, The Tulane University of Louisiana, New Orleans, La. Cloth, 713 pp. \$7.50. Lea and Febiger, Phila., Penn.

In this book the author has set himself a two-fold task: to systematize what is known about the causes and origins of disease, and to present a systematic approach toward influencing the operation of effective causes in the direction of

health and away from disease

To this end, after an introductory discussion, he presents the causes and origins of disease under six categorical headings: Inherited Factors; Defects of Nutritive Elements; Exogenous Chemical Agents; Physical Forces and Energies; Processes and Effects of Invading Organisms; Psychologic and Biosocial Factors And Their Effects.

Each section is followed by a discussion of the ways and means of defense. The book is well organized and well and clearly written and may well become a standard reference text on this subject.

Molding and Casting. Its Technic and Application. By Carl Dame Clarke, Associate Professor of Art As Applied To Medicine, University of Maryland School of Medicine. Cloth, 308 pp., 68 figures. John D. Lucas Co., Baltimore, Md.

Among the newer methods applied to the varied problems of medicine is the preparation of moulage models illustrative of various lesions which have proven their value, not only in teaching, but in the specialized fields of the pathologist and the practitioner of forensic medicine.

In this book, in a clear, understandable and comprehensive fashion will be

found a full exposition of the art of molding and casting.

The volume should be of interest and value to the pathologist, the museum curator, and the medicolegal expert as well as to workers in other more or less allied fields where such preparations are useful.

The Essentials of Pathology. By Lawrence W. Smith, M.D., Professor of Pathology, Temple University School of Medicine, and Edwin S. Gault, M.D., Associate Professor of Pathology, Temple University School of Medicine. With a foreword by James Ewing, M.D. Cloth, 886 pp.; 679 figures, 12 colored plates. \$9.00. D. Appleton-Century Co., New York.

This is a book of outstanding excellence. While addressed primarily to the student, it may well be read by the practitioner and laboratory worker and

deserves a place in every working reference library.

The plan of the book is eminently practical and based upon the all-important relation between pathology and clinical medicine, the latter being, after all, only an extension of the former.

Under each subject the discussion appears on the left-hand page, the illustrations on the right. The illustrations are of a high degree of excellence, well chosen and beautifully reproduced. In the main they are microphotographs largely from the personal collections of the authors. There are also twelve excellent color plates.

Following the didactic presentation are case histories illustrating the clinical picture presented by the lesions discussed.

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The text is clear, concise without being laconic, and evidences the practical experience of the authors.

This is a book which can be recommended without reserve. It deserves the cordial reception and wide circulation it will undoubtedly receive.

Textbook of Medical Bacteriology. By David T. Belding, M.D., Professor of Bacteriology and Experimental Pathology, Boston University School of Medicine, Boston, Mass., and Alice T. Marston, Ph.D., Assistant Professor of Bacteriology and Immunology, Boston University School of Medicine, Boston, Mass. Cloth, 43 figures, 1 plate. \$5.00. D. Appleton-Century Co., New York.

In this volume the authors attempt to present a discussion of bacteriology primarily for the medical student and clinician occupying a position midway between the voluminous reference text and the elementary presentation.

In this endeavor they have been highly successful. Throughout the book emphasis is laid upon the relation of bacteria to public health and preventive medicine. This volume can be recommended as an excellent presentation of the subject, clear, comprehensive and eminently practical.

Clinical Roentgenology of The Digestive Tract. By Maurice Feldman, M.D., Assistant Professor of Gastroenterology, University of Maryland; Associate Roentgenologist, Sinai Hospital, Assistant in Gastroenterology, Mercy Hospital, Baltimore. Cloth, 1014 pp., 358 illustrations. \$10. William Wood & Co., Baltimore.

This is an extremely worth while and valuable book which can be recommended without reserve.

It is an exceptionally comprehensive discussion of the roentgenologic study of diseases of the gastro-enterologic tract embodying, not only an ample and well digested experience, but also a thorough survey of the pertinent literature.

The author's stand upon controversial subjects is sane, logical and amply fortified by extensive and correlated experience.

Well organized, well written and adequately indexed this is a book of real value to the gastroenterologist and radiographer. Indeed, the physician in general may read it with profit and add it with wisdom to his reference library. The Pathology of Diabetes Mellitus. By Shields Warren, M.D., Professor of Pathology in the Harvard Medical School; Pathologist to the New England

Deaconess Hospital, the New England Baptist Hospital, and the Huntington Memorial Hospital, Boston; Director of Massachusetts State Tumor Diagnosis Service. With a Foreword by Elliott P. Joslin, M.D. Cloth, Ed. 2, 246 pp., 86 illustrations, 3 colored plates. \$4.75. Lea and Febiger.

This is, with little doubt, perhaps the most comprehensive as well as the most authoritative discussion of the pathology of diabetes mellitus yet gathered

under one cover.

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The background of this book is experience, not merely accumulated experience, but experience critically analyzed and thoroughly digested and correlated with the clinical picture of diabetes. This book will be invaluable to all who are concerned with this disease, the physician, the pathologist, the specialist and the practitioner at large.

This volume deserves a place in every medical library. As is usual, the

volume is excellently printed and beautifully illustrated.

The Vitamins and their Clinical Applications. By Dr. W. Stepp, Director of the I Medical Clinic, University of Munich; Dr. Kuhnan, Director of the Municipal Institute for Balneology and Metabolism, and Dr. H. Schroeder, Associate at the I Medical Clinic, University of Munich. Translated by H. A. N. Bauman, M.D. Cloth, 173 pp. \$4.50. The Wisconson Cuneo Press, Inc. (Obtainable from The Vitamin Products Co., Milwaukee.)

Developments concerning the vitamins have been so numerous and so varied and are to be found in so many varied publications, that it is a matter of some

difficulty for the physician at large to keep pace with them.

He should, therefore, welcome this book which presents in a very practical

way, the present consensus in this field.

Each of the known vitamins is here discussed, presenting in each instance, the history, chemistry, determination, occurrence, manifestations, absorption, clinical application, physiology, preparation and dosage.

The book is clearly written and embodies a wealth of clinically applicable data

and deserves a place in the reference library of every physician.

It is to be regretted that the index is supplied as an insert and not found with the text.

Pathological Technique. By Frank Burr Mallory, A.M., M.D., Sc.D., Consulting Pathologist to the Boston City Hospital, Boston, Mass. Cloth, 434 pp., 14 illustrations. \$4.50. W. B. Saunders Co., Philadelphia.

This book requires no introduction, for where is the pathologist or laboratory worker whose "Mallory and Wright" shows not the scars of long and honored use?

This volume, its successor, in the words of the Preface, "is not an encyclopedia of methods. Instead, it is a selection of those formulas that practical experience has shown to be of value." The three main sections into which the

book is divided discuss: I. General Materials and Histological Methods; II. Special Histological Methods; III. Autopsy Methods, including Preservation of Gross Specimens, Photography, and Addenda of miscellaneous notes.

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If there should be anyone unfamiliar with the practical value and general utility of the former "Mallory & Wright" a glance at the present volume will lead to its acquisition.

Those to whom the former book is familiar—and their name is legion—will find this volume a worthy successor. It should be in the reference library of every pathologist, laboratory, and laboratory worker.

A General Textbook of Nursing. By EVELYN C. Pearce, Sister Tutor, The Middlesex Hospital etc. etc., Examiner for the General Nursing Council for England and Wales, Examiner in Fever Nursing and Epidemiology for The Diploma in Nursing, London University. Cloth, 888 pp., 174 illustrations. E. P. Dutton & Co., New York.

This is not only, as it is described, "A comprehensive guide to the final state examinations" but also one of the best books of its kind which this reviewer has been privileged to see.

Concise without being laconic, clear, understandable, well organized and well written, it bears the stamp of comprehensive and digested experience. It is, indeed, refreshing to read a nursing text which is neither hyper-technical nor, on the other hand marred by technical "baby-talk," for if the raw material and the product of the present nursing curriculum is what it portends to be there is little excuse for the c-a-t, cat approach all too often evident in nursing texts.

To call specific attention to the many excellent features of this book would exceed the space available for review, but mention of one of its most outstanding chapters, on a subject almost never seen in the average nursing text is that on "The Nursing of The Dying and The Care of The Dead." This should be read by every nurse, and by every teacher of nursing as well.

From the Introduction to the final paragraph of this book it is evident that the author is one who not only knows but loves actual nursing, who not only knows what to do, how it should be done and who realizes that comfort of the mind is as valuable as comfort of the body is of nursing importance, but who knows how to impart her knowledge to others.

Here and there some minor criticism could be made but this book can be highly recommended.

Virus Diseases and Viruses. By SIR PATRICK P. LAIDLAW, M.S., B.Ch. F.R.C.P., F.R.S. Cloth, 51 pp. 90 cents. The University Press, Cambridge. The Macmillan Company, New York.

In this volume is presented the Rede Lecture, 1938 in which is summarized and discussed the present concept of virus diseases and viruses.

While this is a subject to which comparatively recent investigations have

added much, much more remains to be done before the viruses and the diseases produced by them are understood.

The summary of what has been done, what is known and the presentation of the theoretical and investigative possibilities, as presented by Dr. Laidlaw furnish an interesting and valuable survey well worth reading. For this book, though small, is comprehensive in its outlook and a scholarly, authoritative discussion, to boot.

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THE PRACTICE OF CLINICAL PATHOLOGY IN RELATION TO MEDICAL ECONOMICS*

THOMAS B. MAGATH

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Since the first annual convention of the American Society of Clinical Pathologists which took place in this city seventeen years ago, it has been a custom for the retiring president to give an account of the state of the nation in so far as the American Society of Clinical Pathologists is concerned. Taking now a cue from the present President who occasionally sits in Washington and who is charged with a similar annual duty, I propose on this occasion to limit my discussion to a few phases of economics which seem particularly important at this time, rather than to give a complete picture of Society activities.

There is good reason why our presidential addresses should have dealt with economic problems; the opening sentence uttered by the first president on his assuming the chair as temporary chairman at the Missouri Baptist Sanitarium at the first annual convention was, "we are assembled today to take counsel together how best to strengthen the status of the clinical pathologist from a scientific as well as the economic standpoint."

There seem to me to be three main problems of an economic nature which confront pathologists and which do not directly affect the rest of the medical profession, or perhaps I should say, these problems affect the pathologists more particularly than the other specialists or general practitioners, because no major problem can affect one group without having a profound effect

^{*} Received for publication May 27, 1939.

Presidential Address, read before the American Society of Clinical Pathologists at its eighteenth annual convention, held at St. Louis, Missouri, May 11 to 13, 1939.

on all others. I should like to discuss these problems in the reverse order of their importance and the first of these is the relation of the State Board of Health laboratories to practitioners of clinical pathology.

LABORATORIES OF STATE BOARDS OF HEALTH

Since pathology as a medical specialty is but a scant forty years old in this country, there can be no doubt that boards of health started laboratory service long before it was generally available from private sources. But the purpose of the state laboratory was always clear and from the beginning was limited to the control of those diseases which are contagious, in particular tuberculosis, typhoid fever and malaria, and which might affect the general population; and the control of those basic matters which affect large groups of citizens who might be made ill from public supplies of water, food or milk or the disposal of garbage or sewerage. There is not now, nor never has there been, any question as to the right, desirability or necessity of state and federal governments operating in these fields and any group of executives, laboratory men and laymen can quickly come to a conclusion as to where the limits of such control and responsibility lie. knowledge advanced, chiefly by the observations of private practitioners and those in private institutions, a number of other general responsibilities, such as the keeping of vital statistics, care of certain phases of maternal and infant welfare and the supervision of industrial hygiene, were recognized as belonging to governmental agencies but in these the laboratories were not in a key position.

Whatever may have been the motive or the propriety of the matter, as laboratory procedures increased, the state departments kept pace and one finds today that the amount of laboratory diagnosis and investigation conducted in these boards of health has expanded to such an extent that in many states few if any private practitioners of laboratory medicine can exist. In some states the situation is in a vicious circle as clearly illustrated in my own. In Minnesota there are no laboratory physicians practicing except those who derive the major part of their income from an institutional connection and but little private laboratory

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work is done in the state. Since the state laboratory performs such a large share of the laboratory work, no private pathologist can get a foothold and, since there are no strictly private pathologists in the state, the Board of Health has to continue to carry on the necessary laboratory diagnosis in the state.

There are two distinct philosophies which apply to laboratory work performed by the state as to all other governmental services, the first capitalistic, which takes the position that no one should be entitled to free service unless unable to pay for it, and the other socialistic, which predicates that the state should offer its services to rich and poor alike. Most pathologists are evidently distinctly capitalists without benefit of capital, for they strongly believe that except for those laboratory services a state board can render in the control of contagion affecting the public health, state laboratories should be distinctly limited to investigative problems in keeping with a reasonable budget.

But if pathologists are going to hold to such views they will have to solve many problems of their own. First of all, they will have to be able to render service to all who want and need it and at a fair price or, I should say, a price the public can pay. The cost of performing a single Wassermann or other serologic test in a modern state board of health is not more than a few cents and yet a group of our members have strenuously objected to a candidate becoming a member because he is willing to perform such tests for comparable rates.

But supplying pathologists to cover the work of the country on a private practice basis is at the present, and for years to come, an impossibility. There are vast areas in the United States where no pathologist exists for hundreds of square miles and there is not likely to be one there for years to come. Even in the enlightened state of ten thousand lakes there is only one or maybe two pathologists willing to run serologic tests with the exception of those at the University Hospital and at one large private clinic, which does no laboratory work except on its own patients. Where, for example, can a farmer get water from his well analyzed in Minnesota and where, save at the state board of health, can he get a typing of sputum done on his sick child, if he lives in any place other than in one of a half dozen cities or

towns? If pathologists in any state have worked out a complete scheme for taking care in private laboratories of all the laboratory work needed, as efficiently or one half as cheaply as the state board is now doing, I have never heard of it. The pathologists of Minnesota tried most of one night to do it, and gave it up as a bad job.

The most serious hurdle to jump is that of responsibility. The state board is charged by law, and rightly so, with the control of contagious and venereal diseases and no administrator can or should delegate this responsibility to any but those over whom he has absolute control and power to hire and fire. I, therefore, seriously question whether any possibility exists of more than a very minor transfer of duties from the state board of health laboratory to private laboratories.

All this does not mean that I advocate unlimited development of laboratories of state boards of health, for I believe on the contrary that they should be curtailed, but I am trying to point out the simple and painfully apparent fact that until pathologists are able to offer a practical solution for the absorption by private laboratories of the laboratory practice of medicine in a state, it will do little good to rail at the present conditions. tion must include the assurance that the standard of practice will be at least as good as that performed by the state and that the records will be accurate and available for officials who will after all have the problem of following up the patients where public health is at stake. Finally, the private pathologist will have to be willing to report those unfavorable findings on his income-paying clientele which will result in their being held in quarantine against their wishes with the resulting desire to go to a pathologist whose eyes are not so keen and whose conscience is not so sensitive. The problem is not, as you see, just one of having the state laboratories turn over their work to private laboratories.

HOSPITAL LABORATORIES

For many years hospitals have been gradually changing their viewpoint in regard to their functions in a community and their relation to the care of patients. Many have moved a long way

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from the old idea of a "nursing hotel" for the sick. Not only have they established large and important research laboratories but they have to a great extent begun the practice of medicine and the selling and manufacturing of drugs. Two important branches of medicine, radiology and pathology, have been considered by hospital managers as well within their province to exploit to their own interest if not entirely to the interest of the patient. There is a fundamental difference of opinion between the practitioners of these specialties and those hospital managers and associations who would take over responsibilities they cannot rightfully assume. The board of trustees of the American Hospital Association states that "provision of medical service in hospitals is part of the responsibility of the hospital." Apparently a large number of hospital managers believe that "the laws regulating the practice of medicine by corporations do not apply and were never intended to apply to hospitals" and they naïvely dodge the issue by declaring that "the performance of diagnostic and therapeutic procedures consists of practice of medicine in hospitals—not the practice of medicine by hospitals."

Because there are some parts of the practice of pathology and radiology which require physical equipment and mechanical procedures these fields were quickly included in the so-called service of a first-rate hospital and second-rate and third-rate work is sometimes charged for at first-rate prices. In many hospitals the profit from these services has been sufficient to make the superintendents willing to fight to retain the services and they have even branched out and offered the service to physicians in the community. In a great number of instances these services have consisted in the performance of tests done by a technician usually with more nerve and ignorance than common sense and knowledge, or by some young and inexperienced physician who has hopes of using this means of acquiring enough funds to do something better.

If my summary of this situation seems to be in error perhaps I can defend it by the following facts: All technicians certified by the Registry are pledged never to act as a director of a labora-

tory and from the number who are registered and the nature of the registered technicians, there are not enough well trained technicians left to account for the number of hospital laboratories in this country. Again there are 6,166 hospitals approved by the American Medical Association and there are probably not more than 900 physicians trained in pathology in the United States. Of these, approximately 125 may be regarded as nonpracticing teachers and at least seventy-five are in executive and research positions. This leaves about 700 pathologists for the more than 6,000 approved hospitals, to say nothing of the unapproved ones. It takes but little knowledge of mathematics to see that only one hospital in about eight has the exclusive service of a trained pathologist. One hears much today about the great danger from the flood of immigrant physicians into this country and it has been pointed out that many of them are entering the field of pathology. I have no absolute statistics on the matter and I am in thorough accord with the notion that before these unfortunate ones should be permitted to practice in America it should be clearly ascertained whether or not they are competent and are of good moral character, but I cannot, in fairness, maintain that the field of pathology is saturated and that there is no room for competent men in the specialty; nor can I honestly state that American pathology would suffer from the entrance into this country of a Wassermann or a Virchow.

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As a passing thought it may be interesting to recall the condition for approval of hospitals as set down by the American Medical Association. "A physician-pathologist qualified by training and experience to supervise the laboratory service and interpret tissue pathology should be employed on a full time or part time basis. When this is not practicable, arrangements should be made with a consulting pathologist for tissue diagnosis, postmortem work and the interpretation of the more complicated tests and determinations in clinical and surgical pathology, as well as in general clinical laboratory work. Preferably the pathologist should be a physician who holds a certificate of the American Board of Pathology." The same rule in general exists in the requirements for approval of a hospital by the American

College of Surgeons, yet evidently many institutions have been approved without this requirement having been met. It is by no means clear to me just how approval of some of these hospitals is secured, for on the basis of a table published in the March 11, 1939, issue of the Journal of the American Medical Association there were only 4,673 departments of pathology in the 6,166 approved hospitals and these were directed by only 3,601 medical graduates. One can readily conclude that only about one-fifth of these have qualifications equal to those required for certification by the American Board of Pathology.

From these observations it must be apparent that many hospitals, even approved hospitals, have inadequate laboratory services and this in itself presents a challenge to pathologists who are members of our society and ought to be a mild warning to hospitals. But I am not at this point interested in just this phase of the problem. With the almost universal clamor for hospital and medical insurance and the laborious and heartrending pleadings of the professional social welfare workers, a renewed interest has been aroused over the laboratory and x-ray equipment. I say equipment advisedly, because to date little interest seems to have been stimulated among the welfare workers in the qualifications of the personnel. Hospitals are trying generally to include in the insurance fee the guarantee of laboratory and roentgenologic service. This has led to more commercialization of these subjects and unless the specialists arouse themselves quickly, the standards of the profession will be seriously lowered, the quality of the work sacrificed and the public will suffer accordingly. It is at least somewhat of a satisfaction to see that at last the interest of the medical profession, at least that of the American Medical Association, has been mildly stimulated, not out of any particular desire, I imagine, to defend these two specialties but because of the realization that if pathology and radiology can be practiced by hospitals acting as corporations, there is nothing to prevent these institutions from hiring gynecologists, ophthalmologists, internists and even surgeons, on the same basis, and running a complete commercial medical service with dire consequence to the profession and the public.

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With a view to trying to bring some practical solution to the relation of pathologists and hospitals a set of agreements has been drawn up; if this is put into practice, it will do much to bring about a stoppage of many abuses now prevalent. The preamble makes clear the situation and reads as follows: "It is recognized that pathological and other laboratory services are essential elements in the diagnosis and treatment of disease and in its prevention and control; that a competent laboratory service under skilled direction is an essential element in the hospital; that the number of qualified pathologists is limited; and that many hospitals and communities are too small to maintain locally qualified specialists in this field."

The meat of the agreement so far as we are concerned is to be found in the paragraphs dealing with professional personnel and these I quote: "Hospital services in pathology and other branches of laboratory work should be organized as a department, under the direction of one or more competent pathologists who should be responsible for all the laboratory services of the hospital. Such a chief or chiefs of department should be a physician and wherever possible, should be one who is a qualified specialist in pathology, preferably a diplomate of the American Board of Pathology or the equivalent body in Canada. If, because of the size or isolation of the hospital or for other reasons, a qualified pathologist is not available locally, some member of the general medical staff, trained in pathology or paying particular attention to the subject, should be appointed in immediate charge of the department. Under these conditions a consultation service should be arranged for the department with a qualified pathologist or with another hospital or agency in which a qualified pathologist is in charge of the laboratory service." All of these and the other provisions have been carefully considered and are recommended for adoption both by pathologists and hospital superintendents.

The method of handling laboratory charges in the numerous insurance policies will require much careful consideration, but unless all medical services are to be included in the insurance schemes, the laboratory service should be excluded also and under no circumstances should it be included as a nonmedical service.

THE PRACTICE OF CLINICAL PATHOLOGY BY UNQUALIFIED PERSONS

To my mind the most important problem in the economic aspect of clinical pathology is the decision as to who shall be permitted to practice the specialty. So far as physician-clinical pathologists are concerned the problem may be said to be well on the road to solution if indeed not, to all practical purposes, solved. A Board of Pathology has been created which has to date certified 689 pathologists as qualified. While there are some certified who perhaps should not have been, and others not certified who should be, no fair-minded person can help but admit that within a few years it will be possible to examine a published list and find in it 95 per cent of those pathologists who, judged by their peers, are capable of practicing pathology in a manner at least equal to any specialty practiced by any other group of medical specialists. In short, the pathologists have washed, dried and ironed their own linen, dirty or clean, as you might have thought it.

But there remain two other groups of persons who practice clinical pathology who have not even begun to air their linen either publicly or privately. The first, physicians not particularly trained in pathology; the second, laymen. It is perhaps not difficult to understand why physicians in general should practice, to a limited extent at least, this branch of medicine. Usually it is for financial reasons or convenience or from lack of access to the services of a pathologist, but that they should consider themselves competent is beyond the understanding of one who for a quarter of a century has made it his almost undivided business to study and practice the specialty, and who after that period of time realizes the hopelessness of trying to master more than a small part of the whole subject.

There is perhaps a very definite reason why the internist feels competent "to run a laboratory." In most schools, clinical pathology is taught in the department of medicine and the student naturally comes to look on clinical pathology as a part of medicine

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and not of pathology, a subject frequently only associated with tissue diagnosis and the dead house. Usually an internist limits himself to procedures other than those involving morphologic pathology and this strengthens the conclusion. Now in so far as the physician limits this form of diagnosis to his own endeavors on his own patients no great harm is done to anyone. If he is busy he can't do much of it and if he's not, he won't. But soon he becomes tempted to branch out. He hires a technician, usually a nice looking one, turns over the whole laboratory to her, along with his telephone, x-ray machine, electrocardiograph, and social engagements and then, not yet satisfied, begins to "take in" outside work. The reports are made by the technician and the consultations as well. It might be worth while to suggest that some correction of this unsatisfactory condition may be had by conference with the qualifying Board in Internal Medicine and by making some effort to induce medical schools to transfer clinical pathology to the department of pathology, a more suitable and useful department.

Far more important just at present is the practice of pathology by laymen, consisting of certain graduates in the basic sciences, especially chemistry, and laboratory technicians. In recent times there has developed a vociferous minority in a certain section of the American Chemical Society which has made an issue of the position taken by our Society that clinical pathology is a specialty of medicine and that to practice clinical pathology is to practice medicine. The matter has come to a head in Pennsylvania where the State Board of Medical Education and Licensure has rightly held that to operate a clinical laboratory is to practice medicine and to practice medicine one must obtain a medical license. The Chemical Society has challenged the ruling and has gone back to the agreement made in 1924 between a committee of the American Medical Association and committees from the American Chemical Society and the American Association of Pathologists and Bacteriologists. This it considers a Magna Charta and states it will resist all effort to abrogate it.

It is well to know the background of the resolution. The President of the American Medical Association was authorized at

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that time by the House of Delegates to appoint a committee to confer with representatives of the two aforenamed societies. The joint committee met and adopted the agreement which in turn was adopted by the House of Delegates, without conferring in any way with the Section on Pathology and Physiology and our society, which at that time included men who were performing probably in excess of 90 per cent of all medical laboratory work being done at the time, excepting that done by state boards of health. The two groups the American Medical Association took into the Munich pact were never and are not now particularly concerned with the actual practice of clinical pathology as a part of the practice of medicine. The American Association of Pathologists and Bacteriologists is made up in the main of teachers and nonmedical men and has on several occasions made it plain that it is not concerned with the economic aspects of pathology or bacteriology. The American Chemical Society can boast of only a handful of so-called medical chemists in comparison with its huge membership. The representatives from the American Medical Association were not known as private practitioners of laboratory medicine.

From the time of the passage of the agreement the action of the officials of the American Medical Association and the House of Delegates has been in marked disagreement with the contract and in 1930 the Council on Medical Education and Hospitals published the following rule concerning clinical laboratories: "The director of an approved clinical laboratory should be a graduate of an acceptable college or university of recognized standing, indicating proper educational attainments. He shall have specialized in clinical pathology, bacteriology, pathology, chemistry, or other allied subjects for at least three years subsequent to graduation. He must be a man of good standing in his profession." In 1936 the Council "deprecated" the operation of clinical laboratories under lay direction.

Last year the American Chemical Society procured the services of an attorney in the matter and has definitely started a campaign calculated to permit laymen, in particular chemists, to operate and direct clinical pathologic laboratories. So far the

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character of the published attack takes two forms: first, that of the lawyer, arguing in typical manner the legality of the case. His brief contains matters probably new to him but old and readily conceded by even the novice in the field of medicine, but matters of no moment in the particular problem; there is a more or less skilled dodging of the real issue at stake and no evidence that the lawyer knows or is willing to admit the fundamental nature of the practice of medicine. The second form is exemplified by the statements of the paid executive secretary of the organization and his outpourings are caustic, sarcastic and bombastic. There is no real scientific argument in them and the main theme is developed from the alleged mercenary attitude of the American Society of Clinical Pathologists and the alleged desire of the American Medical Association to obtain a monopoly in the field of diagnostic laboratory work.

An answer of a sort was contained in an unsigned editorial in the Journal of the American Medical Association, an editorial which, from the nature of the third sentence, apparently was written by a youthful member of the staff who could not, because of lack of experience, possibly have grasped the significance of the basic problem; it is not "principally a commercial problem ancillary to the practice of medicine" and the method of attack by the American Chemical Society is not the important issue at stake. Having one's toes stepped on is not, after all, crucial. It is a bit difficult to see why the answer by the American Medical Association did not take a similar form to that made some years ago when it played a successful part in stopping a prominent mail-order house from examining urine sent in by mail. The argument was based on the sound principle that all branches of medicine should be practiced by medical graduates who should take the responsibility for the findings.

The fundamental question is this: Is the practice of clinical pathology a part of the practice of medicine? When this is once settled, all other questions can be automatically answered. Now, to be sure, members of this society have no doubt as to the correct answer to this question, but I wonder if we are not still thinking in terms of the early years of our specialty. Have we not heard it

so often said that the pathologic laboratory was to be used as an adjunct to medicine that we have never thought to inquire as to whether the statement was wholly true?

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Let us consider an item or two in relation to this. Show me a clinician who can absolutely diagnose by any method except by laboratory procedures a case of leukemia, agranulocytic anemia, amebiasis, almost any worm infection, or a host of other diseases. Does not the laboratory establish, indeed diagnose, these conditions without any reference to history or physical findings? Does anyone doubt that the presence of myelocytes and other immature cells of this series in predominant numbers establishes a positive diagnosis of myelogenous leukemia regardless of a clinician's opinion? Do you know of any condition when the finding of Mycobacterium tuberculosis or Mycobacterium leprae fails to establish a diagnosis even in the absence of clinical findings? Is there a clinician living who can recognize early pregnancy from clinical observations as accurately as a clinical pathologist can determine it with 10 c.c. of urine? Determine a blood sugar value of 125 mg. per 100 c.c. or more after a twelve hour fast or 180 mg. after ingesting two doses of 50 gm. of glucose one half hour apart and you have diagnosed a case of diabetes regardless of an Osler's opinion to the contrary.

And so one could go on indefinitely with examples galore and still not mention the fact that many tissue diagnoses are unpredictable by any clinician or surgeon; instead of confirming a diagnosis the examination of the tissue often refutes one or establishes an entirely unsuspected condition. The prime function of a medical laboratory is to diagnose disease and next to guide treatment. Need I mention reticulocyte counts following liver therapy, or prothrombin determinations after the administration of vitamin K?

It can be seen that the argument put forth by the American Chemical Society, that the clinical laboratory is not a diagnostic agency, of itself is fallacious. That organization takes the position that the laboratory is merely a mechanical shop in which analyses are made, in purely a mechanical and routine fashion. There is no denying the fact that many procedures can be con-

ducted in the laboratory in a routine fashion. The American Society of Clinical Pathology must never get itself into the position of asserting that one without a medical training could not analyze and analyze correctly many biological materials, but this is only dodging the real issue. A laboratory that is to be of service to the attending physician and patient must do more than merely make analyses according to some routine procedure. It must make these analyses in an understanding fashion and be prepared to give an adequate explanation and statement of the significance of the results; in short, the laboratory must furnish competent medical consultation. But the laboratory must go farther than this. Its personnel must frequently obtain, by surgical procedures, material from the patient for analysis and it must constantly be contributing to the knowledge of medical science. It must protect the patients by being included in the "privileged communications" law. No laboratory is worthy of the name that does not fulfill all of these functions and this society is on perfectly sound ground in insisting on it.

The American Chemical Society uses another form of argument which is based on a fallacious concept, to the effect that any and all medical men are required by law to be able to interpret all laboratory tests. The experience of any clinical pathologist who has practiced his profession for six months will disprove that contention. The best trained clinicians in the world are the ones who most frequently consult pathologists, and the law has never required that physicians should bring to bear on a case any but ordinary skill and judgment, and ordinary skill and judgment do not at the present time imply that the physician shall know the entire field of laboratory medicine.

The idea that a chemist or bacteriologist or physicist is competent to direct a comprehensive clinical laboratory is absurd on the face of it, for the clinical laboratory embraces so many more fields than these limited ones that even the man trained in medicine and especially trained in the basic sciences finds it difficult enough to keep reasonably well abreast of the developments. The brief submitted by the lawyer of the chemical society im-

plies that the chemist is perfectly competent to make a diagnosis by bacteriologic means and to make tissue and necropsy examinations but, of course, such a contention is hardly worth serious consideration. The American Society of Clinical Pathologists does not deny that the clinical chemist or bacteriologist has a definite place in the clinical laboratory. It believes that place, however, is under the supervision of a licensed physician who has been especially trained in laboratory medicine. The society believes that the chemist and bacteriologist should be encouraged to seek positions in clinical laboratories but it does maintain that when either the health or life of a patient is at stake, or both are at stake, the diagnosis, procedure, interpretation and relevant advice should be supervised and given only by a competent licensed physician and this I believe to be all that is necessary to be said on the subject.

For a long time it has been quite apparent to those who have studied the situation as to the origin of the entire difficulty in the development of the laboratory conducted by the layman, that the responsibility for the condition rests almost wholly on the clinician and surgeon. If clinicians and surgeons did not send patients to laymen for examination there would be no problem of any kind in this connection and the solution to all of this lies entirely in the hands of the medical man. It seems strange that, with all of his fighting for the absolute control of the field of medicine, he should cast all of his arguments aside when he has a patient who is in need of special laboratory service and should turn such a patient over to anyone claiming to be a laboratorian without even taking the trouble to find out the qualifications of such an individual. I firmly believe that the only way this whole matter may be successfully solved is to educate the clinicians and surgeons to the importance of protecting their own reputations and the health of their patients by seeing to it that the laboratory procedures which they order are executed under the supervision of, and that they are interpreted by, a competent pathologist.

This very conclusion, however, places on the pathologist an

extremely important and serious task. A part of this task has already been nearly completed, that of certification and making sure that those who call themselves pathologists are properly trained and are of good ethical character. The next and huge task is to provide sufficient numbers of well-trained pathologists to cover the demand adequately. This, I believe, can be done with the co-operation of the organized medical profession, but without it the finest development of clinical pathology in this country cannot be achieved.

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THE PREPARATION OF ANTI-M AND ANTI-N TESTING FLUIDS*

I. DAVIDSOHN AND I. ROSENFELD

From the Pathological Laboratories of the Mount Sinai Hospital, Chicago, Illinois

Landsteiner and Levine^{2, 3} discovered in 1927 the existence of two new agglutinogens in human red blood cells, one of which or both together are present in the blood cells of all persons. They were designated by the discoverers as M + N -, if the former was present and the latter absent, as M - N + if the reverse was the case, and as M + N + if both were present. At present a simplified nomenclature is generally accepted: M, N, and MN. The term "blood types" is applied to these new properties, the term "blood groups" being reserved for the properties A. B. AB. and O. The essential difference between the blood groups and the blood types is that there are specific agglutinins in human serum capable of clumping red blood cells of groups A. B. and AB. while no such agglutinins exist for the blood types M, N, and MN which is the reason why the discovery of the blood types took place in a roundabout way. Serums of rabbits injected with human blood of one individual, when absorbed with the blood of another individual, were capable of clumping the red cells of some persons and not of others, irrespective of their blood groups. Similar methods led to the discovery of several other specific blood properties, but they have not been studied sufficiently and will not be considered here.

The properties M, N do not change during life. They are fully developed in the newborn and were found in embryos from $1\frac{1}{2}$ to $2\frac{1}{2}$ months old. In embryos from 4 to 6 months old they were estimated to be of equal strength as in adults, in that

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respect differing from the properties A and B, which are weaker in infancy and in early childhood than in adults.

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The properties A, B are present in all tissue cells and in many secretions. The M and N properties could not be reliably demonstrated outside of the red blood cells. The distribution of ag-

TABLE 1
DISTRIBUTION OF BLOOD TYPES M, N, AND MN IN RELATION TO BLOOD GROUPS

BLOOD GROUP	NUMBER	PER CENT	TYPE	NUMBER	PER CENT	FREQUENCY ACCORDING T WIENER
						per cent
AB	20	6.9				
			M	7	35	
			N	6	30	
			MN	7	35	
A	95	32.8		NA.		1
			M	36	38	
			N	13	14	
			MN	46	48	
В	47	16.2				
			M	23	49	
			N	7	15	
			MN	17	36	
0	128	44.1				
			M	40	31	
			N	21	17	
			MN	67	52	
				Sun	nmary	
			M	106	36	30.25
			N	47	17	20.25
			MN	137	47	49.50
Total	290	100.0		290	100	

glutinogens M and N shows less pronounced racial differences than do agglutinogens A and B, but further studies are urgently needed.

Table 1 shows the distribution of the blood types in our series of 290 determinations. The differences in relation to the blood groups can be explained by the small numbers and is supported by the fairly close agreement of the totals at the foot of the table

with the figures quoted by Wiener¹⁰, page 169. Lower values for N and higher values for M were found in Indians, and the reverse was reported for the Ainu's in Japan.

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Landsteiner and Levine⁵ showed that the agglutinogens M and N are inherited as Mendelian dominants. They assumed the existence of a single pair of allelomorphic genes, M and N, and of three genotypes MM, MN, and NN with three corresponding phenotypes, M, MN, and N. It is apparent from the above that there can be no absence of any of the two properties: M -N -. The medicolegal application of the hypothesis of Landsteiner and Levine increased greatly the possibility of an exclusion in suits for alleged paternity, as will be brought out later.

The preparation of anti-M and anti-N immune serums and testing fluids is a fairly difficult process and requires a great deal of patience, thoroughness and experience, acquired only by careful application. The testing fluids are made by removing the non-specific agglutinins by means of graded absorptions. Mechanical work can never be successful in this field. must learn to evaluate a variety of factors in order to get reliable and trustworthy results. Some of these factors are: The marked variation in the sensitiveness of the red blood cells of different individuals, the variations in the strength of the immune serums and of the testing fluids, the effect of temperature, etc. Once good testing fluids are on hand the determination is not difficult, although not quite so simple as the determination of the blood groups. On the other hand it would be desirable if more pathologists would interest themselves in this work because it seems reasonable to hope that eventually more states will follow the example of New York and Wisconsin and will adopt blood grouping tests as a routine procedure in cases of disputed paternity.

The purpose of this paper is to present our experiences in the production of anti-M and anti-N immune serums and in the preparation of testing fluids. Many different methods have been suggested in the literature, each claiming advantages of its own. We do not intend to review all the methods, but we shall limit ourselves to a presentation of those which we tried out personally

and to a discussion of our results.

TECHNIC OF IMMUNIZATION

Two methods were used: 1) Wiener's method¹¹: Daily intravenous injections of washed red blood cells of type OM or ON beginning with 0.1 cc. and increasing by 0.1 cc. until the dose of 0.5 cc. is reached. Seven days after the fifth injection the rabbits are bled from the ear vein, the blood serum is absorbed with a mixture of blood cells of types OM, A_1M , and BM or ON, A_1N , and BN, to determine the suitability of the serum for preparation of testing fluids. If the serum is found satisfactory, the rabbit is exsanguinated. Usually several courses of immunization were necessary. The intervals were one or more months. This detail differed from the original recommendation of Wiener of seven days' rest periods between each course of injections.

2) Levine's method⁶: An intravenous injection of 0.2 cc. of washed blood cells of type OM or ON is followed five days later by a subcutaneous injection and again two hours later by an intravenous injection of 0.2 cc. Three further subcutaneous and intravenous injections are administered at five day intervals, until five intravenous and four subcutaneous injections were given. The preliminary bleeding is done seven days after the last intravenous injection. Wiener's method will be referred to in this paper as the rapid method and Levine's as the slow method.

Table 2 summarizes the results achieved with the two methods of immunization. Twelve rabbits were injected with blood OM. The key for the evaluation of the results is given at the foot of the table. The slow method gave poor results in six rabbits but after one month's interval, a course of daily injections according to the rapid method resulted in an excellent immune serum in two rabbits and in good serums in three. One rabbit died.

The rapid method gave poor results in six rabbits, a second course after one month's interval resulted in an excellent immune serum only in one rabbit, while in the remaining five the results remained poor. A third course of daily injections after a second interval of two months gave poor results in one rabbit and a good immune serum in another. The last rabbit (no. 15) was bled only partially and was given a rest of two months, after which a series of daily injections produced an excellent immune serum.

Eight rabbits were treated with ON blood. In four the slow method was used with excellent results in two, with good in the other two. Of four rabbits treated according to the rapid method one produced a good anti-N serum after a single series, another gave poor results after the first series, and a good immune serum following a second course of daily injections after a rest of three months. That same rabbit was rested for two more months, then given five daily injections and a good immune serum resulted. Two rabbits died in the course of treatment.

It is generally accepted that only OM or ON blood be used for the immunization of rabbits. The effect of administration of blood OMN was tested. Two courses of daily injections at one month's interval failed to produce either an anti-M or an anti-N immune serum, but after another month's rest a third

TABLE 2

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	PROL	Production of anti-M immune serums			P. P. B.	PRODUCTION OF ANTE-N IMMUNE SERUMS	
Rabbit	Immun- ized with	Method used	Results	Rabbit	Immun- ized with	Method used	Results
. 5	MO	Slow	Poor	7, 10	NO	Slow	Excellent
2, 3, 6	MO	Slow	Poort	8, 9	NO	Slow	Good
11. 13. 14	OM	1 month interval then rapid Rapid	Good	19	NO	Ranid	Poor
1		1 month interval then rapid	Poor			3 months interval then rapid	Good
12	OM	Rapid	Poor			2 months interval then rapid	Good
		1 month interval then rapid	Poor	20	NO	Rapid	Good
		2 months interval then rapid	Poor				
15	OM	Rapid	Poor	23, 26	OMN	Rapid	Poor
		1 month interval then rapid	Poor		OMN	1 month interval then rapid	Poor
		2 months interval then rapid	Good		ON	1 month interval then rapid	Good
		2 months interval then rapid	Excellent				
16	OM	Rapid	Poor				
		I month interval then ranid	Excellent.				

Key for reading of results:

* Excellent: Grossly visible (at least ++) clumping in a mixture of 1 drop of absorbed serum, 1 drop of physiologic † Good: Grossly visible (at least ++) clumping in a mixture of 2 drops of absorped serum and 1 drop of the cell sussolution of sodium chloride and 1 drop of the cell suspension.

pension.

‡ Poor: When clumping was grossly not discernible in above dilutions of serum.

course of daily injections of blood ON led to the production of a good anti-N serum.

It seemed to us that the slow method was more satisfactory for the production of good anti-N immune serum, one course being usually sufficient to produce good or even excellent results, while a combination of the slow method followed by the rapid after an interval of one month seemed preferable for the anti-M immune serums.

Preparation of testing fluids

The serums as obtained from immunized rabbits are not suitable for diagnostic purposes because they contain in addition to the type specific agglutinins, species specific agglutinins and react with cells of all types and groups. The latter must be removed by absorption with suitable blood cells.

The process of absorption is a very delicate procedure crowned with success only when patience is paired with meticulous care. The reliability of the determination of blood types is conditioned upon the use of satisfactory, sensitive, and specific testing fluids which should be kept in mind to be successful in this work.

Fresh immune serums must be inactivated before absorption, after several weeks' stay in the ice box inactivation is not necessary.

Preparation of anti-M testing fluids

The rabbit serum is diluted with from fourteen to twenty-nine parts of physiologic solution of sodium chloride according to its titer. Mix equal volumes of centrifuged blood cells of types ON, A₁N and BN. Add to the mixture three volumes of physiologic solution of sodium chloride, mix, centrifuge for ten minutes at moderate speed, discard supernatant fluid and repeat the washing and centrifuging two more times. After the third washing the cells can be used for absorption provided the supernatant fluid was not hemolyzed. We found that when the cells without the plasma are added for the preparation of the mixture, hemolysis was less frequent than otherwise. If hemolysis was present after the third washing, the cells were not suitable for absorption.

To one volume of the serum dilution one-half volume of packed red cells of the mixture was added drop by drop, shaking after each drop, and then every fifteen minutes, while the test tube was kept for one hour at room temperature. After centrifuging the supernatant fluid was removed and tested for non-specific agglutinins. If they were present, the absorption had to be repeated usually with one-quarter volume of the cell mixture. In most anti-M serums a single absorption was sufficient.

Preparation of anti-N testing fluids

These are more difficult to prepare on account of the readiness of the specific anti-N agglutinins to be removed together with the non-specific anti-M agglutinins when treated with M blood. It is recommended to carry out the

absorptions at 37°C. with a mixture of cells of types OM, A₁M and BM. Frequently two or even more absorptions with smaller amounts of cells are necessary for the complete removal of the nonspecific agglutinins.

Titration of testing fluids

Two per cent suspensions of washed and packed cells of following blood types are used: A_1M , OM, BM, AMN, A_1N , ON, BN. One of the three specimens of blood group A must be of subgroup A_2 . Variations according to the available blood types and groups are permissible as long as the blood groups A_1 , A_2 , B, O, and the blood types M, N and MN are represented. The MN is essential particularly for the testing of anti-N serums because the N factor in combination with M is known to be considerably less sensitive than when alone, thus being a good indicator of the sensitiveness of the serum. The factor M is also less sensitive in MN than when alone, but the difference is less marked.

To the first of two rows of seven small test tubes (75 x 12 mm.) two drops of the absorbed rabbit serum are added to each test tube, and one drop of serum followed by a drop of physiologic solution of sodium chloride to each tube of the second row. This gives in the second row a dilution twice that of the first. A drop of each of the seven cell suspensions is added to the test tubes in both rows according to the markings. The test tubes are kept at room temperature for two hours and shaken vigorously during that time every fifteen minutes. The reading is done with the naked eye and interpreted as described in table 3. When negative to the naked eye, it is checked with the low power (32 mm. ob-

jective) of the microscope.

Table 3 presents a few typical records. Serum 1-M (anti-M) was excellent for use after a single absorption, serum 5-M required a second absorption with one-four a volume, because after the first absorption bloods of type N were still clumped. Serum 15-M showed after the first absorption the same degree of clumping for cells M and N. Such serums are rarely satisfactory even after further absorptions because the specific agglutinins are removed together with the non-specific. In this case another series of injections after a rest period led to the formation of a satisfactory immune serum. Serum 12-M was unsatisfactory, because the titer of the specific agglutinins was too low to permit a safe differentiation. As can be seen in table 2, that serum failed to improve even after further immunization. The two examples of anti-N serums demonstrate the necessity of a greater number of absorptions as compared with the anti-M serums, although we seemed to have less difficulty in the preparation of anti-N testing fluids than most of the authors who wrote about it. We had the impression that the addition of the blood cells drop by drop to the serum dilution facilitated the absorption.

The titers of the testing fluids were determined according to the results of the titration. Only those serums were used of which at least two drops agglutinated distinctly and specifically the corresponding red cells, but did not

TABLE 3 EPARATION OF ANTI-M AND ANTI-N TESTING FLUIDS

					TITRATIC	TITRATION WITH KNOWN CELLS	VN CELLS		
BERUM	ABSORPTION WITH RED	RE-ABSORPTION WITH RED BLOOD CELLS	WO	AıM	BM	BMN	N ₂ N	NO	BN
NORDER			*+++04	40+++	+++0	40+++ 40+++ 40+++ 20+++, 20-	20-	-02	-02
1-M	A ₁ N, BN, ON		+++0+	40+++ 40+++ 40+++ 40++	40+++	40++	20+,	+0+	40++
2-M	A ₁ N, BN, ON	4 volume AiN, BN, ON 40++	40++	40++ 40++	40++	40+	20 - 40++	20-	20-
15-M	A ₁ N, BN, ON		++0+	40++			-06	20-	20-
12-M	AIN, BN, ON	20 4 volume A ₁ M, BM, OM 20-	88	20-	20∓ 20∓	20+++,	20+++, 40+++		40+
N-01	A ₁ M, BM, OM	y volume A ₁ M, BM, OM 20+,	20+,	20+,	20+,	40+++	20+++, 20+++, 20+++	40++	0+++, 20+++ 40++ 40++
		volume A ₁ M, BM, OM 20-	1 20-	20-	20-	40++	20++,	20+++,40++	+0+1

Key for reading of results: ++++, one large clump; +++, several large particles; ++, small clumps readily seen with the naked eye; +, only microscopically visible clumping; ± microscopically indistinct clumping; -, no clumping even micro-* The titers are the reciprocal values of the dilutions of the testing fluids.

scopically.

T gs

agglutinate the cells of the other type even when examined with the microscope. These testing fluids were classed as good. When one drop of the testing fluid gave distinct clumping when diluted with a drop of physiologic solution of sodium chloride, it was labeled as excellent.

Testing of unknown bloods for M and N

Approximately a two per cent suspension of red blood cells is used, prepared by adding one drop of whole blood to 1 cc. of saline or of 1 drop of packed cells to 2 cc. of saline. Specimens of known blood groups and types must be included as controls. Three anti-M and three anti-N testing fluids are used. At the same time the blood group is determined by using three undiluted human serums of subgroup A1 with a high titer of anti-B isoagglutinins, and two undiluted human serums of group B with a high titer of isoagglutinins anti-A. As an additional means of detecting group A and of determining the subgroups A₁ and A2 we employ immune serums of rabbits treated with boiled sheep blood.1 A lower dilution as determined by titration is capable of clumping distinctly the red cells of subgroups A1 and A2 while a higher dilution clumps only the red cells of subgroup A1 and not those of A2. A drop of the unknown blood cell suspension is added to each test tube and a drop of the known blood cells to the controls. The blood group should be checked in each case by testing the serum against red cells of known groups. The tubes are shaken vigorously. We believe it advisable to shake the test tubes after cells were added to each row and not to wait with the shaking until the blood cells were added to the tubes of the entire rack. The test tubes are kept at room temperature for two hours and shaken vigorously at frequent intervals. The reading is done as described above.

The results for all eighteen blood group and type combinations as copied from our protocols are recorded in table 4.

The use of boiled human red blood cells for the preparation of testing fluids

One of the difficulties in the preparation of testing fluids is the need of relatively large quantities of blood cells of the different blood group and blood type combinations. Some of them, e.g. BN, are quite rare and therefore not easy to get. Testing fluids do not keep as well as the undiluted and unabsorbed immune serums and therefore it is advisable to prepare a supply for only a few months. It would be very desirable if the cells could be preserved for longer periods than is possible with the known methods, e.g. that of Rous and Turner⁸: Three parts of sterile whole blood are mixed with two parts of 3.8 per cent sterile solution of sodium citrate and five parts of 5.4 per cent sterile solution of glucose. The citrate and glucose are prepared and sterilized separately and mixed immediately before use. In this mixture the blood keeps very well in a good ice box from three to four weeks and occasionally even longer. We were able to keep blood in satisfactory condition for as long as six weeks.

TABLE 4
DETERMINATION OF THE BLOOD GROUPS, SUBGROUPS, AND OF THE BLOOD TYPES

		TIPE		M	Z Z	Z	WN	MN	M	N	Z;	Z	MIN	N N	12	MN	M	Z	MN	BLOOD CELLS OF KNOWN GROUP	M	NN:	ZZ
		GROUP		A ₁ B	A ₂ B	Alb	A ₂ B A ₁ B	A_2B	A ₁	A2	\mathbf{A}_1	A2	A1	A ₂	a a	a m	0	0	0	BLOOD C KNOWN	A2	A_1	9 O
SERUM	A-II-A	97.	1:40	++++	1	++	++	1	++	1	++	1	+	1	1	-	. 1	1	1		1	++	1 1
IMMUNE	ANT	01.1	1:10	+	+:	+-	+ +	+	++	+:	+++	+ -	+++	+			ı	ı	ı		+	++	1 1
	B (anti-A)			++++	+ :	+ -	+ +	+	+++	+ :	+ -	+ -	+ :	++++		1	1	١	1		++	++	1 1
M8	B (a	4	ms	++	+ -	+-	+ +	+	++	+ :	+.	+ -	+ :	++	1	1	1	1	1		++	++	1 1
HUMAN BERUMS		m	Undiluted serums	++	+ :	++-+-	++	++	1	1	1	1	ı	1 -	+ +	- +	- 1	1	1		1	1	+ 1
HU	A ₁ (anti-B)	69	Und	++	+ + + + + + + + + + + + + + + + + + + +	+ -	++++	++	1	1	1	1	1	1 -	+ +	+	- 1	1	1	CONTROLS	1	1	+ 1
	A	1		-			+++++++							1 -					ı	CONT	1	1	+ 1
N	NI-III	a	1:30	1	1	+ -	++	+	1	1 :	+++	+ -	+ -	+	++	- +	- 1	+	++		1	++	++
-	reging from anti-in	10	1:20	1	1 -	+ - + -	++	++	1	1	+ -	+-	+ :	++++	11	- +	- 1	+	++		1	++	1+
C. A. american	PRILING	2	1:40	1	1		+++														1	++	++
N. m.	- TIN	16	1:40	++++	+++	1	1 +	+++	+++	+++	1	1]	+ -	+ -	+ 1	++	+++++++++++++++++++++++++++++++++++++++	- 1	++		++	+-	+
M. seems of the state of the st	a prome a	64	1:20	++			1 +	-	-							++	++++	- 1	+++		+++	++++	+
		1	1:40	+++	+++	1	+++	++	+++	+++	1	1 -	+ -	+ +	- 1	++	- +	- 1	+++		+++	+++	+ 1

i s i s v t

We studied the effect of boiling on the agglutinogens M and N. If boiling did not destroy them, a very convenient method would be available for keeping blood for preparation of testing fluids.

Specimens of blood cells of groups A₁M, BM, and OM were washed separately twice with physiologic solution of sodium chloride. Equal volumes of the packed cells of each group were mixed and the mixture was washed once more. A twenty per cent suspension was prepared from the packed cells with physiologic solution of sodium chloride and boiled on the water bath for thirty minutes. The same procedure was followed for cells A₁N, BN and ON. The suspensions of boiled cells were used for the preparation of testing fluids. The amount of cells required for the absorption was calculated, the suspension was centrifuged until the cells were well packed, the supernatant fluid was completely removed with a capillary pipette and the diluted serum was added. The cells were thoroughly mixed, shaken, and the absorption carried out as was already described. The anti-M serums were absorbed with the N cells at room temperature, the anti-N serums were absorbed with the M cells at 37°C.

According to table 5, the ability of agglutinogen N to remove the anti-N agglutinins was considerably decreased by boiling, although a certain amount of absorption took place. On the other hand, the agglutinogen M proved highly thermostable. Boiled cells kept in the ice box for six months were equally suitable for the preparation of anti-N testing fluids as fresh blood cells. We have adopted that procedure and find it very helpful.

We are carrying out further experiments with cells of type N to see whether boiling for shorter periods than thirty minutes may have less deleterious effect and at the same time be sufficient to preserve them for long periods.

Preservation of immune serums and of testing fluids

Different procedures have been recommended. We found that several of the recommended preservatives have a deteriorating effect upon the titer.

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We bleed the rabbits from the heart, using sterile needles and syringes, and we attempt to maintain strict asepsis during the entire handling of the serum. We store it without preservative in 1 cc. sterile glass vials. The testing fluids are also kept without preservative. Occasionally a deposit forms in them, but we did not notice any influence upon the agglutination. Preservatives had in our experience a very destructive effect, occasionally even after surprisingly short periods. It is of course imperative to observe as strict asepsis as is possible. The use of boiled blood for the preparation of anti-N testing fluids makes it easier to eliminate contamination. The immune serums and the testing fluids are kept in a reliable refrigerator at about 6°C. We found that freezing is a very efficient method for the preservation of anti-M and anti-N immune serums and of testing fluids. The serums were placed in a refrigerator at -8°C. and were kept frozen for from six to seven months. They were thawed out after that period. The immune serums were used for the preparation of testing

Preparation of Anti-M and Anti-N Testing Fluids with Raw and Boiled Human Red Blood Cells Comparison of results TABLE 5

ERTM		ARRORRED WITH		RE-ARGORRED WITH		TITRATION	WITH KNO	fiteation with enown red blood crils of types:	OD CELLS OF	F TYPES:	
					МО	A ₁ M	BM	BMN	A ₂ N	NO	BN
	a volu	y volume of blood cells Raw	Raw								
1-M	1-M A1N, BN, ON	3N, ON		0	+++0+	40+++ 40+++ 40+++ 40++	+++0+	++0+	20-	-02	-02
1-M	"	93	Boiled		40++	40+++ 40+++ 40+++	40+++	40+++	40+	40+	40+
				volume of blood cells							
				AIN, BN, ON	++0+	40++ 40++ 40++ 40+	40++	+0+	+0+	+0+	40+
16-M	,	3	Raw		+++0+	40+++ 40+++ 40+++ 40++	40+++	40++	20+,	20+,	20+,
									-04	40-	40-
				t volume of blood cells	1 1 0	1 1 07	1	- 109	8	06	00
	77	73		AIN, DIN, ON	+++0#	1105 1105 1105 1105	1 1 10	1 - 05	1 0 0	107	100
10-M			Bolled		40++	++07 ++04 ++04 ++04	++0+	++0	+0++	++0+	40-
				y volume of blood cells	100						
				AIN, BN, ON	40++	40++ 40++	++0+	20+	40+	+0+	20-
8-N	a volu	8-N 4 volume of blood cells Raw	Raw	4 volume of blood cells	D0						
	A ₁ M,	A ₁ M, OM, BM		A ₁ M, OM, BM	-02	-02	-02	40++	40++	40++	40++
Z-8		"	Boiled	"	20-	-02	20-	40++	40++	40++	40++
10-N	"	7,7	Raw	"	20+	-02	20+,	40++++40+++40+++40++	-40+++	+++0++	++0+
							40-				
				"	-02	-02	-02	20++	40++	40++ 40++ 40++	40++
10-N	"	"	Boiled		40+	20+,	20+,	40+++	40+++	40+++40+++40++	++0++
						40-	40-				
				22 22	-02	-02	-02	40++	40++	40++ 40++ 40++	40++

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fluids and were found to be very satisfactory. Equally satisfactory were the testing fluids which were kept frozen for six months. This method ought to prove very useful because it permits doing away with preservatives and eliminates entirely the effect of bacterial contamination.

How to send specimens of blood by mail for determination of the M and N agalutinogens

Wiener¹⁰ (page 124) recommends the following procedure:

About 2 cc. of blood are obtained from the vein under aseptic precautions. One-half of it is added to a vial with an appropriate amount of the solution of Rous and Turner, the formula of which was mentioned; the other half is placed in a vial without any preservative. From a baby, one can obtain the necessary blood from a deep puncture in a heel into a few 10 cm. long segments of narrow glass tubing (2 mm. outside diameter). They are filled about two-thirds with blood and sealed at both ends in the flame. Careful labeling is essential. The obtaining of at least two specimens from each person is an additional check against errors in labeling. The tubes and vials should be well packed in cotton and sent by air mail and special delivery to shorten the time in transport as much as possible.

Identification of persons who submit to blood tests

Cases are on record where persons with a guilty conscience sent substitutes for examinations. To avoid possible criticism it is advisible to take finger-prints of adults and footprints of babies prior to the examination.

What can be expected from blood grouping in cases of disputed paternity?

When a child is born in lawful wedlock and the husband denies paternity, when a child is born out of lawful wedlock and the man named by the mother as its father denies paternity, when newborn infants have been accidentally or wilfully interchanged and it is desired to identify the parents of the child, and finally when a woman pretends that a certain child is her own, there is an opportunity to apply the blood grouping tests to exclude paternity and occasionally also to exclude parentage of a woman. The two basic rules which express the application of our knowledge of inheritance of blood groups are:

1. The agglutinogens A and B cannot appear in the blood of a child unless

present in one or both parents.

2. A parent of group AB cannot have a group O child and a group O parent

cannot give rise to a child of group AB.

While the first law is accepted by all authorities without reservation, there is still some difference of opinion concerning the second. Some authors grant it the same validity as the first, while others claim that in view of one apparently well supported exception, it should be interpreted as a strong evidence that paternity is extremely unlikely.

Table 6 lists the groups of children that cannot be the issue in a given parental combination. If found they indicate illegitimacy.

Thomsen, Friedenreich and Worsaae⁹ formulated a theory of inheritance of subgroups A_1 and A_2 , which adds further possibilities toward exclusion of paternity. According to their concept, A_1 is dominant over A_2 , and A_1 and A_2 are dominant over O. They formulated the following rules:

1. A₁ cannot appear in a child unless present in the blood of one or both parents.

TABLE 6
THE LANDSTEINER BLOOD GROUPS IN PARENTS AND CHILDREN

	GROUPS OF PARENTS	GROUPS OF CHILDREN NOT POSSIBLE
1	oxo	A, B, AB
2	OXA	B, AB
3	OXB	A, AB
4	AXA	B, AB
5	AXB	
6	BXB	A, AB
7	OXAB	O, AB
8	AXAB	O
9	BXAB	O
10	ABXAB	0

TABLE 7
THE AGGLUTINGGENS M AND N IN PARENTS AND CHILDREN

	TYPES OF PARENTS	TYPES OF CHILDREN NOT POSSIBLE
1	MNXMN	
2	MNXN	M
3	MNXM	N
4	MXN	M and N
5	NXN	M and MN
6	MXM	N and MN

2. The combinations, A₁B parent and A₂ child and vice-versa cannot occur.

3. In the matings A₁B and B, and A₁B and A₁B, A₂B children cannot result. Extended studies suggest that Thomsen's theory is probably correct but it is advisable to use it merely as corroborative evidence.

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The rules of inheritance of the agglutinogens M and N as laid down by Landsteiner and Levine⁵ have been fully corroborated by large numbers of reports. They are:

1. Agglutinogens M or N cannot appear in a child unless present in the blood of one or both parents.

2. A type M parent cannot give rise to a type N child and vice-versa a type N parent cannot give rise to a type M child.

The published exceptions to these laws are explained by illegitimacy.

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TABLE 8

CHANCES OF PROVING NON-PATERNITY WITH THE BLOOD GROUPS (WHERE PUTATIVE FATHER HAS BEEN FALSELY ACCUSED)

Per cent exclusions; putative father in group

0	A	В	AB
23.5	7.7	14.6	39.9

Chances of proving non-paternity with the agglutinogens A, B, M and N

GROUP		0			A			В			AB	
Туре	M	N	MN	M	N	MN	M	N	MN	M	N	MN
Chances (per cent).	50.0	54.6	23.5	39.6	45.1	7.7	44.1	49.3	14.6	60.7	64.3	39.9

Modified from Wiener, A. S., Blood Groups and Blood Transfusion. Charles C. Thomas, Springfield, Illinois, pages 192 and 197, 1935.

TABLE 9
PATERNITY CASES IN NEW YORK CITY

YEAR	TOTAL CABES HANDLED	TOTAL SETTLED	TOTAL SUIT	JUDGED*	DENIED†	MARRIED	DISC CASES‡	PENDING
1930	842	349	564	366	61	16	112	31
1931	1016	576	534	333	69	28	92	43
1932	1226	635	707	430	90	34	121	75

^{*} In these cases, the defendant was adjudged the father of the child by the court.

† In these cases the defendant was acquitted.

‡ This includes cases which were amicably settled, or where the child is adopted or died, or moved out of the jurisdiction.

§ Pending cases are those which are being followed up: cases are followed until the child is 16 years of age.

Modified from Wiener, A. S., Blood Groups and Blood Transfusion. Charles C. Thomas, Springfield, Illinois, 1935, page 199.

Table 7 lists the illegitimate children on the basis of the M and N blood types. How much the chances of exclusion of paternity have been increased by the application of the tests for M and N agglutinogens can be seen from Table 8.

That there is a great field for frequent application can be seen from Table 9.

SUMMARY

Our experiences in the preparation of anti-M and anti-N immune serums and testing fluids were presented. The details of the technic that we found useful were given.

Boiled blood of type M (OM, A₁M and BM) was found very well suited for the preparation of anti-N testing fluids. We believe that it can replace entirely the use of raw blood of type M. On the other hand, for preparation of anti-M testing fluids, raw blood of type N must be used because the latter does not resist boiling.

Anti-M and anti-N immune serums and testing fluids were kept frozen and without preservatives for over six months without the slightest trace of deterioration. The value of determination of the blood types M, N, and MN for exclusion of paternity was discussed.

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TOXICITY AND THERAPEUTIC EFFECTS OF SULPHAPYRIDINE IN ANIMALS*

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* Received for publication April 10, 1939.

The following studies were made with the idea of obtaining (a) information on the toxicity of sulphapyridine ("2-(para-aminobenzenesulphonamido) pyridine"—Whitby, "Dagenan"—Merck) on mice, (b) its therapeutic effect on type three and six pneumococcus peritonitis in rats. Sulphapyridine according to Whitby¹ has the following formula:

$$NH_2$$
 SO_2 NH N

TOXICITY EXPERIMENTS WITH SULPHAPYRIDINE IN WHITE MICE

To determine the toxicity, four groups of white mice, each group consisting of four animals and each animal weighing approximately 25 grams, were fed every day with the average therapeutic dose in group A; ten times the therapeutic dose in group B; 50 times in group C, and 100 times in group D. By assuming that 4 grams of sulphapyridine is the average maximum therapeutic dose for a human weighing 75 kilograms, we arrived at the therapeutic dose for mice, as follows: One gram of body weight would receive 4 divided by 75,000 grams of sulphapyridine. As the average mouse weighs 25 grams the therapeutic dose for a mouse would be 100 divided by 75,000 grams, or 1.4 milligrams. A suspension of 1 gram of sulphapyridine in 50 cc. of distilled water was made and thoroughly shaken. 7/100 cc. of this suspension contained 1.4 milligrams, or a therapeutic dose. This amount was administered orally every day to each of four mice over a period of fourteen days by means of a calibrated pipette. In a similar manner four mice in group B were treated. Two tablets of sulphapyridine equal to 1000 milligrams were dissolved in 5 cc. of distilled water thus obtaining 14 milligrams in 7/100 cc. or ten times the therapeutic dose. In group C, each of four mice received 50 times the therapeutic dose over a period of fourteen days. Two thousand milligrams of sulphapyridine were suspended in 10

cc. of distilled water so that 0.35 cc. contained 70 milligrams, i.e., 50 times the therapeutic dose.

In group A, B, and C, the administration of the drug was simple because the suspension was not too concentrated and the quantity to be given not too large. None of the twelve mice thus treated died or appeared to be sick, judging by their appearance and behavior. Two of each group were sacrificed by means of chloroform, the remaining six were alive eight weeks after the last dose was administered.

In group D we tried to administer 140 milligrams per mouse per day. Two thousand milligrams of sulphapyridine were suspended in 5 cc. of distilled water so that 0.35 cc. of the suspension contained 140 milligrams. The suspension was concentrated, nevertheless during the first day all four animals, which had not received any fluid for the twelve hours preceding the administration of the drug took the entire amount, but we had to give small feedings every three hours. During the next few days we were not able to force the animals to take the entire amount; we succeeded, however in giving them not less than sixty times the therapeutic dose and frequently close to one hundred times per day. One of the animals expired on the third day after having received three hundred ten times the therapeutic dose. The animal choked to death in an attempt to feed the drug. The other three survived for seven days of treatment, no. 2 having received a total of 680 milligrams which is approximately 68 times the therapeutic dose. No. 3 received 760 milligrams or 76 times the therapeutic dose, animal 4 a total of 720 milligrams or 71 times the therapeutic dose. Animal 2 was sacrificed by chloroform. The remaining two were alive eight weeks after discontinuance of the drug, and showed no ill effects. None of the three animals treated showed any signs of toxicity as judged by their behavior and appearance during the seven days in which the drug was administered.

In group E, three animals were treated with a lethal dose which, according to Whitby, is 16.6 milligrams per gram or 415 milligrams per mouse. We dissolved 2000 milligrams in 5 cc. of distilled water and tried to give each animal 1 cc. of the suspension. One animal had to be omitted as it would not take any fluid when held. Animal 1 received a total of 520 milligrams in three days, then expired. Animal 2 received 600 milligrams in two days and expired. Both animals appeared drowsy, the fur was rough, and they were unable to keep their balance. Both these animals were subjected to necropsy.

The six animals in group A, B, and C, the two animals in group D, and the two animals in group E, showed no naked eye changes at necropsy. Lungs, heart, liver, kidney, spleen showed no histological changes. Table 1 summarizes the results.

Effects of sulphapyridine on type three pneumococcus peritonitis in rats

Twelve white rats, each weighing approximately 125 grams, were injected intraperitoneally with a 24 hour broth culture of type three pneumococci. The pneumococci were obtained from a patient suffering from a severe type three

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pneumococcus pneumonia. Temperatures were taken every three hours during the day. When the temperature, taken rectally, reached 102°F. (approximately 24 hours after injection), treatment was instituted, giving to nine of the animals 7 milligrams of sulphapyridine every three hours for three doses per day. We arrived at 7 milligrams of sulphapyridine as the therapeutic dose for a 125 gram rat by assuming again that 4 grams is the therapeutic dose for a human weighing 75 kilograms. The drug was given orally in an aqueous suspension and measured with a calibrated pipette. Three animals were not treated. All died thirty hours after the injection. Necropsy revealed purulent peritonitis. Peritoneal and heart cultures showed a profuse growth of pure type three pneumococci which gave specific type swelling of the capsule. Of the treated group, two

TABLE 1

dnoup	1	2	3	4	5	6	7	8	9	10	11	12	13	14	TOTAL
A. 4 animals with therapeutic dose	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	19.6
B. 4 animals with 10 times the therapeutic dose	14	14	14	14	14	14	14	14	14	14	14	14	14	14	196
C. 4 animals with 50 times the therapeutic dose	70	70	70	70	70	70	70	70	70	70	70	70	70	70	980
D. 4 animals with	140	90	80												310
100 times the		80	70	80	70	140	100								680
therapeutic		80	90	60	140	140	110								760
dose		90	80	140	80	100	80								710
E. 3 animals with	40	Omi	tted-	-would	d not	take	dose								40
lethal dose	220	100	200		1										520
	200	400													600

^{*} Died.

animals were sacrificed and subjected to necropsy two days following the inauguration of treatment. Their temperatures had fallen from 102.8 and 103 respectively to a normal level after four doses of sulphapyridine. Necropsy revealed a mild peritonitis and a few organisms in the heart's blood and peritoneal cultures, both giving specific type capsule swelling.

On the third day after the commencement of the drug, two animals died. Their temperatures had remained high, i.e., above 102. Necropsy revealed purulent peritonitis with fecal material in the peritoneal cavity. Examination showed perforation of the intestines by the injecting needle. Culture of heart's blood and peritoneal contents revealed many type three pneumococci and B. Coli. Some of the pneumococci gave specific type swelling while others showed degeneration of the capsule. The temperatures of the remaining five

animals had reached a constant normal level after the fourth or fifth dose of sulphapyridine. On the fifth day treatment was stopped and two more animals were sacrificed the same day. The peritoneum showed no naked eye changes and culture of the heart's blood and of the peritoneal cavity showed only a few pneumococci in culture. The heart culture was sterile. The pneumococci no longer gave specific swelling of the capsule. The remaining three animals were sacrificed on the eighth day. Heart and peritoneal cultures were sterile. Throughout the experiments three control animals showed a temperature ranging between 97 and 98.2.

SUMMARY GROUP I

1. Twelve rats were injected intraperitoneally with 1 cc. of a 24-hour broth culture of type three pneumococci obtained from a blood culture of a patient.

2. All twelve animals developed fever ten hours after injection.

3. Nine animals were treated with 7 milligrams of sulphapyridine every three hours for three doses per day after the temperature had passed 102.

4. Three animals were untreated. All died approximately

30 hours after injection.

- 5. In the treated group two animals died. Necropsy performed immediately after death showed secondary invasion by B. Coli in the peritoneum and heart's blood due to trauma of the intestines.
- 6. The remaining animals were sacrificed at different intervals after instituting treatment. Cultures revealed that the decrease in the degree of peritonitis and bacteremia until cure was directly proportionate to the length of time the animal was treated. Table 2 summarizes the results.

Effects of sulphapyridine on type six pneumococcus peritonitis

The investigation was carried out in identical circumstances as described in the experiment with type three pneumococci. In this instance 1 cc. of a 24 hour broth culture of type six pneumococci obtained from a patient dead of pneumococcus meningitis, was injected intraperitoneally. Twelve white rats each weighing 125 grams were injected. All developed fever approximately 10 hours after injection. Nine animals were treated with 7 milligrams of sulphapyridine orally every three hours after the temperature had passed 102 degrees (approximately 24 hours after injection of the pneumococci). Three were untreated. All three died approximately 30 hours after injection. Necropsy

TABLE 2
TEMPERATURES IN DEGREES FAHRENHEIT

			peri- type			pool		nitis	o u	Culture in the	mulg.	acri-	gross feart cul-							
			Necropsy revealed purulent peritonitis. Culture of heart's blood and toneum showed a profuse growth of pneumococci, giving excellent specific swelling			Necropey revealed a mild peritonitis, and a few organisms in the heart's blood and peritoneal cultures, both giving type specific capsule swelling		nimals expired. Necropsy revealed a purulent foul-smelling peritonitis with feeel material in the peritoneal eavity. Culture of heart's blood and	peritonies contents reveal many pineumococci and b. 5011, some of the pineu- mococci still gave type specific swelling, while others show degeneration of the capsule	Both animals sacrificed. Necropsy revealed no gross pathology. Culture of heart's blood and peritoneal cayity show only a few organisms in the	perioneal cuture, pneumococci giving no longer type specine sweiling. Heart culture was sterile	All 3 animals sacri- ficed. Necropsy	revealed no gross pathology. Heart and peritoneal cul-	rures sterile						
H		*	of her			nism		foul	b. co	ross / a fe	ar ty	926	926	926						
8ти рат		4 10 1 4 10 1 4 10 1 4 10 1 4	nococ			orga		Cul	le ot	ou luo	Jong	972	974	976						
8 _T		10	Cult			a few		pur vity.	whi	aled	on .	974	120	978 978						
H		4	of p			and g typ		ad as	lling	reve	MINI	86	978							
7TH DAY		-	itoni			nitis,		veale	y pn	al ca	130 130	978	974	976						
72		10	t per			oth g		y re	ecific	Necro	noco	26	97	974 970						
17		4	rofus			ild pe		crops n the	eveal pe sp	d. 1	prile	976	26	974						
OTH DAT		-	a p			la m		Ne lial	nts r	rifice	peritoneal culture, pneu Heart culture was sterile	16	978	86						
\$		10	ecropsy revealed toneum showed specific swelling			ealed real c		Animals expired.	l gav	sac lood	re w	971	973	970						
H		4	y rev			y rev		exp scal 1	peritonesi c mococci still the capsule	imah rt's b	cultu	926	126	86						
STH DAY			rops			ad pu		mals ith f	00000	h an	eart	126	926	978						
20	Hour	= Z	Nec			Nec		Ani	484	Bot	ă#	*26	972	974						
ы		+	the	the	the	ificed	ificed			88X	97. X	97 X	974 X	973 X						
TE DAY		-	Animal died 30 hours after the i njection Animal died 31 hours after the injection		s after	Animal sacrificed at 10 a.m.	Animal sacrificed at 10 a.m.		104°	973 X	6X	973 X	88x	8×						
		10	ed 30 hours i njection	d 31 hour injection	1 294 hour injection	Anim at 1	Anim at 1	103* X	104x	X8	6X	97 X	2×	978 X						
×		+	ied 30	inje	inje	978 X	978 X	103. X	103s X	97°	97°	97.X	97. X	978 X						
3RD DAY		-	p la	p la	al die	Animal died 294 hours after the injection	al die	al die	al die	al die	al die	8×	100x	1028 X	103 X	88X	SX.	8X	188 X	98x
38		10	Anim	Anima	Anim	80 X	101 X	102 X	1031 X	100x	90X	8X	95X	100 X						
_		-	at n.	104° at	at m.	1018 X	102 X	101 X	103 X	102 X	1018 X	101 X	102°	1018 X						
2ND DAY		-	103* Died at 4 p.m.	103* 10 Died at 5 p.m.	1041 Died at 3:30 p.m.	102°	1024 X	103 X	102s X	102s X	103 X	102s X	1034 X	103°						
61		10	1028	1024	102*	102s X†	103 X	1033 X†	102s X†	1024 X	102s X†	1024 X	103 X	103°						
		4	101	1008	101	101	1018	101	1008	*66	100	*66	1001	1008						
IST DAY		-	186	-86	*86	86	186	280	888	86	186	186	986	*86						
18		10.	974	26	86	97	974	26	974	971	86	26	972	926						
ANI-	MUM-	BER	H	п	H	IV	>	IA	ИИ	VIII	IX	×	XI	ТХ						

Temperature taken on 3 control animals ranged between 97 and 98?

* All animals given 1 cc. of a 24 hour broth culture of type III P neumococci intraperitoneally at 10 a.m. the first day.

† The animals received at this time 7 mgm. of an aqueous solution of sulphapyridine orally.

All animals given 1 cc. of a 24 hour broth culture of type III Pneumococci intraperitoneally at 10 a.m. the first day.
 The animals received at this time 7 mgm. of an aqueous solution of aulphapyridine orally.

TABLE 3
TEMPERATURES IN DEGREES FAHRENHEIT

IST DAT	2		R	ZND DAY		20	3RD DAY		47	TH DAY		25	STE DAT		91	6TH DAY		7TH DAY	AY	87	STH DAY	
											14	Hour										
		-	10	-	*	10	-	*	10	1	+	10	1	4	10	-	4	10 1	1 4	10	-	+
0	£26	866	102•	1031	104	Anin	al die	ed 31 hour injection	Animal died 31 hours after the injection	after	the	Nec	ropey	reve	aled nts s	pouru	is pr	Necropsy revealed purulent peritonitis.	itis.	Cult	re of	ecropsy revealed purulent peritonitis. Culture of heart's blood and peritoneal contents and peritoneal spread a profuse growth of pneumococci, giving excellent
-	8	101	102*	104		Anin	in di	ed 28 hou injection	Animal died 28 hours after the injection	after	the	3	ne ed	OCI III	S W C	900	1 170	type special swelling of the department	9			
	66	101	102*	1044		Anin	and die	d 284	Animal died 284 hours after the	after	the											
	86	1013	1021 X	102×	162 X	8×	ž×	103	Animal died approx. 30 hrs. after B was	diedap. 30 hrs. B was	ap- hrs. was	N P P P	ood a	nd pu dege	aled eriton nerat	o noi	rulent onten f cap	Necropsy revealed a purulent foul-smelling peritonitis. blood and peritones contents showed many pneumocoshowed degeneration of capsule, while others showed B. coil	wed r	ng p	pneur pneur s sho	ecropsy revealed a purulent foul-smelling peritonitis. Culture of heart's blood and peritoneal contents showed many pneumococci—some of which showed degeneration of capsule, while others showed good swelling—and B. coli
	8	1008	102 X	8 X	103°	101 X	&×	%X				Both	anir bear	t's bl	Both animals sacrificed, the heart's blood and p	nd pe	Necr	opey en co	revea	ed a	slight	oth animals sacrificed. Necropsy revealed a slight peritonitis. Culture of the heart's blood and peritoneal contents revealed a few pneumococei, many
	•86	101	1024 X	103x	102s X	8×	*×	×97				0 0	the ca	pecil	9	dunie	73 10 5	8	ente,	WDIII	ocue	giving specine awening of the capsure, while others showed degeneration of the capsule
1	8	101	102s X†	103x	101 X	SM	×8	2×	97. X	%×	878 X	Both	anir	nals 's blc	Both animals sacrificed.	heed.	Nec	ropsy	reve	led r	few i	oth animals sacrificed. Necropsy revealed no gross pathology, Culture of heart's blood and peritoneum showed very few organisms in the blood;
	100	101	108 X	102s X	101x	SX	8×	×3	5X	\$×	16 X	Des	peritone	5	nan	8761	ne.	menz	10000	E .	Nu V	peritones, culture sterne. Freumococci giving no fonger type specing swelling
	186	101:	102s X†	8 X	101 ×	8×	%×	XX.	2X	×	25 X	978	874	86	881 8	974 978	m	Both animals sacrificed, gross pathology., Cult	imals	sacri	floed.	oth animals sacrificed. Necropsy revealed no gross pathology. Culture of heart's blood and
	*66	101	102°	103°	102 X	8×	100 X	%×	28×	978 X	878 X	974	88	1.46	82.6	97 973	12	peritoneum sterile	neum	steri	•	
	100	1008	102s	8×	108 X	8×	8×	%X	×8	S×	×8e	*26	86	186	97.	97. 98	-	974 974	97.	97.	97.6	98 Both animals sacri- ficed. Necropsy
	:06	101	108 X	108x	102 X	2×	%×	%×	76X	×97	8×	188	97.	970	86	97.	97e 97e	978	97.	86	978 978	path. Culture of heart's blood and

Temperature taken on 3 control animals ranged between 97 and 98?

• All animals given 1 cc. of a 24 hour broth culture of type VI Pneumococci intraperitoneally at 10 a.m. the first day.

† The animals received at this time 7 mgm. of an aqueous solution of sulphapyridine orally.

revealed purulent peritonitis. Culture of the heart's blood and peritoneal contents showed a profuse growth of pneumococci giving specific type swelling of the capsule. One of the treated group died approximately 30 hours after the administration of the first dose of sulphapyridine. Necropsy showed injury of the intestine caused by the injecting needle and peritonitis. Culture of heart's blood and peritoneal contents showed many B. Coli and pneumococci. Some of the pneumococci gave good swelling of the capsule with type six serum while others showed degenerative changes in the capsule. The remaining eight treated animals showed a drop in temperature to normal after the fourth or fifth dose of sulphapyridine. They were sacrificed at different intervals after inauguration of treatment and again showed that the decrease in the number of pneumococci in the peritoneum and heart's blood, until cure, was directly proportional to the length of time the animal was treated. Table 3 demonstrates the exact results.

CONCLUSIONS

- 1. We believe the drug to be comparatively harmless and non-toxic in large doses in mice.
- 2. We believe the drug has definite curative properties in experimentally induced pneumococcus peritonitis types three and six in rats, when administered sufficiently early and in adequate amounts.

REFERENCE

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SERUM PROTEINS, TAKATA-ARA REACTION, AND LIVER FUNCTION TESTS IN LYMPHOGRAN-ULOMA VENEREUM*

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Lymphogranuloma venereum is a chronic infectious and contagious disease characterized by local lesions and widespread systemic manifestations and caused by an ultra-microscopic filterable virus. Infection is venereally spread. One of four initial inoculatory lesions is encountered. The usual one is a superficial ulceration on the external genitalia in the male, or on the posterior vaginal wall in the female, and is followed by characteristic pathological lesions in the regional lymph nodes and the surrounding connective tissue. Furthermore, probable widespread dissemination of the virus occurs with the production of pathological sequelae both locally and generally. Diagnosis can usually be made by the use of the Frei reaction, or by the more or less characteristic pathological picture presented in biopsy specimens. Attention has been recently called to the systemic manifestations in the acute and chronic phases 18, 40 of the disease. It is our purpose to present detailed evidence of some of the systemic manifestations of the disease as far as the laboratory studies are concerned.

Probably the most characteristic alteration in the blood of

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these patients is the change in the serum proteins. As early as 1931, Nicolau²⁶ noted an increase in the refractive index of the serum of patients with lymphogranuloma venereum, but he erred in thinking that the albumins were present in greater concentration than the globulins. In 1936, Williams and Gutman⁴¹ reported hyperproteinemia in this disease and called attention to the hyperglobulinemia. They further remarked about the possible relationship of these pathologically hyperglobulinemic sera to the inordinately increased sedimentation rates and the anticomplementary properties of such sera as related to the complement fixation tests for syphilis. Gutman, Gutman, Jilson, and Williams¹² later in the same year, reported a series of 36 cases in which 26 showed hyperproteinemia. The increase in proteins was found to be in the globulin fraction and mainly in the euglobulin fraction. In the same communication they point out an apparent discrepancy in the acid base equivalence of these patients in which acid ions appear to be in excess. This, they feel, is due to an error in the factor used in calculating the base bound to protein in the presence of hyperproteinemia. Gutman and Gutman¹¹ have subsequently introduced a correction factor to compensate for this error insofar as calcium is concerned. Hyperproteinemia has also been reported by Rosen, Rosenfeld, and Krasnow³³; Jones and Rome^{17, 18}; and others in this disease.

While hyperproteinemia, and in particular hyperglobulinemia, is commonly found in lymphogranuloma venereum, it is not unique in this disease, nor has it any semblance of pathognomonicity. The presence of hyperproteinemia and hyperglobulinemia has been reported in multiple myeloma^{33, 34, 31, 29, 27}, cirrhosis of the liver¹⁹, tuberculosis^{22, 23}, kala azar^{20, 36}, schistosomiasis³⁶, leprosy^{8, 33}, Boeck's sarcoid^{210, 42}, wucheriasis⁵, and syphilis⁴³. In regard to this last report, Rosen, Rosenfeld and Lyons³², have found that hyperglobulinemia is not characteristic of syphilis.

MATERIALS AND METHODS

In the past two years we have studied 79 patients on whom 105 determinations of the serum proteins have been made. These studies were done in the following manner:

The blood was a fasting specimen, collected with minimal stasis, transported to the laboratory without delay, where the serum was separated from the cells and determinations were made on the same day that the blood was collected, usually within two to three hours following the collection. Proteins were determined by the method of Greenberg and by the method described by Campbell and Hanna.2 These methods were found to agree on several occasions in the highest and lowest values within a range of plus or minus two per cent. The Takata-Ara reaction was determined on the same sample of serum on which the proteins were determined, except in a few instances. Determinations, where abnormal, were repeated on the same sample. Duplicate determinations were done in the detailed analysis of the sera to estimate the possible relation of the euglobulins to the Takata-Ara reaction. A few exceptions to this are noted in the text of the paper. The liver function tests were either done on the same day that the plasma proteins were determined, or within a week afterwards in every case. The only exception to this rule is that the galactose tolerance was usually done one to six days following the serum protein determination and other liver function tests. The determinations of the liver function tests were done in the laboratory of the Gastrointestinal Clinic of the Graduate Hospital. The Takata-Ara test was performed by the technic used in the aforementioned clinic (after Jezler). No attempt was made to classify varying degrees of positivity. A positive test was recorded in the presence of definite flocculation in three or more tubes which did not disappear after twenty-four hours.

PRESENTATION OF DATA

The distribution of the values for the various serum determinations are shown in the figures 1, 2, and 3, and table 1.

In table 1, the figures are arranged in order of the decreasing globulin values simply for the sake of convenience. The highest values of the total protein determination is 13.33 grams per cent, and the lowest 6.02 grams per cent. The average is 8.55 grams per cent.

3 or 2.86 per cent of the determinations lay between 12 and 13 grams per cent; 5 or 4.77 per cent lay between 11 and 12 grams per cent; 10 or 9.52 per cent lay between 10 and 11 grams per cent; 14 or 13.33 per cent lay between 9 and 10 grams per cent; 35 or 33.33 per cent lay between 8 and 9 grams per cent; 24 or 22.86 per cent lay between 7 and 8 grams per cent; and 14 or 13.33 per cent lay between 6 and 7 grams per cent. It will be noted that 73 or 69.52 per cent of the determinations fell within the range of 6 to 9 grams per cent, while 32 or 30.48 per cent

TABLE 1

	NAME	WASSERMANN	TOTAL PROTEIN	ALBUMIN	GLOBULIN	TAKATA
1	N. W.	Neg.	11.20	2.25	8.95	Pos.
2	A. T.	Pos.	13.30	4.50	8.80	Pos.
3	P. W.	Pos.	10.66	3.05	7.61	Pos.
4	E. R.	Pos.	11.20	3.65	7.55	Pos.
5	P. W.	Pos.	10.32	2.92	7.40	Pos.
6	S. A.	Pos.	11.20	3.98	7.22	Pos.
7	N. E.	Pos.	10.80	3.65	7.15	Pos.
8	N. W.	Neg.	10.40	3.32	7.08	Pos.
9		Pos.	10.40	3.65	7.07	Pos.
	A. T.		10.72	3.19	7.05	Pos.
10	P. W.	Neg.				Pos.
11	E. R.	Pos.	9.44	2.52	6.92	
12	W. B.	Pos.	10.40	3.65	6.75	Pos.
13	K. T.	?	12.00	5.31	6.69	Pos.
14	A. B.	Neg.	10.48	3.85	6.63	Pos.
15	C. L.	Neg.	11.20	4.65	6.55	Pos.
16	A. S.	Pos.	9.20	2.72	6.48	Pos.
17	L. S.		8.80	2.39	6.41	Pos.
18	P. W.	Neg.	8.88	2.59	6.29	Pos.
19	C. D.	Pos.	9.12	3.32	5.80	Pos.
20	M. C.	Pos.	8.80	3.05	5.75	Pos.
21	A. J.		12.00	6.31	5.69	Pos.
22	G. S.	Pos.	9.60	3.98	5.62	Pos.
23	E. E.	Pos.	11.20	5.64	5.56	Pos.*
24	R. H.	Pos.	10.44	5.04	5.40	Neg.*
25	A. G.	Pos.	10.00	4.65	5.35	Pos.
26	F. S.	Neg.	6.96	1.73	5.23	Pos.
27	I. P.	Neg.	9.20	3.98	5.22	Pos.
28	L. D.	Pos.	9.52	4.32	5.20	Pos.
29	L. J.	Pos.	9.04	3.85	5.19	Pos.
30	W. M. R.	Pos.	9.28	4.12	5.16	Pos.
31	M. C.	Pos.	8.20	3.05	5.15	Pos.
32	H. W.	Neg.	8.45	3.37	5.08	Pos.
33	K. D.	Pos.	8.00	2.93	5.07	Pos.
34	K. D.	Pos.	8.24	3.25	4.99	Pos.
35	C. L.	Neg.	8.96	3.98	4.98	Pos.
36	J. M.	Pos.	8.84	3.98	4.86	Pos.
37	B. McK.	Pos.	8.16	3.32	4.84	Pos.
38	L. G.	Pos.	8.48	3.65	4.83	Pos.
	J. B.			3.98	4.82	
39		Pos.	8.80			Pos.
40	A. W.	Pos.	9.76	5.05 3.32	4.71	Neg.
41	E. J.	Pos.	8.00		4.68	Pos.
42	G. W.	Pos.	7.60	2.99	4.61	Pos.
43	I. P.	Neg.	8.00	3.42	4.58	Pos.
44	L. T.	Pos.	9.20	4.64	4.56	Neg.
45	T. M. N.	Neg.	8.96	4.45	4.51	Neg.
46	L. D.	Pos.	6.60	2.15	4.45	Pos.
47	K. D.	Pos.	7.76	3.32	4.44	Undet
48	L. D.	Pos.	6.59	2.15	4.44	Pos.
49	F. C.	Pos.	8.32	3.91	4.41	Pos.
50	L. W.	Pos.	8.24	3.85	4.39	Pos.
51	M. H.	Neg.	9.60	5.31	4.29	Neg.
52	L. L.	Neg.	8.24	3.98	4.26	Neg.*
53	D. W.	?	7.68	3.45	4.23	Pos.
54	T. M.		8.32	4.12	4.20	
55	H. W.	Neg.	8.39	4.21	4.18	Pos.

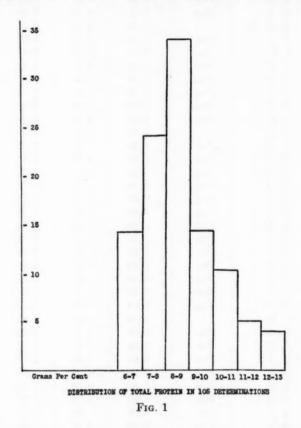
TABLE 1-Concluded

	NAME	WASSERMANN	TOTAL PROTEIN	ALBUMIN	GLOBULIN	TAKATA
56	C. M.	Pos.	8.80	4.65	4.15	Undet
57	I. P.	Neg.	7.92	3.78	4.14	Pos.
58	W. W.	Pos.	9.36	5.24	4.12	Pos.*
59	G. B.	Pos.	8.20	4.10	4.10	Neg.
60	T. S.	Pos.	7.28	3.19	4.09	Neg.*
61	F. R.	Neg.	8.72	4.65	4.07	Undet
62	J. M.	Pos.	7.92	3.90	4.02	Neg.
63	G. W.	Pos.	6.80	2.79	4.01	Pos.
64	H. R.	Pos.	8.00	4.05	3.95	Neg.
65	В. Т.	Pos.	7.20	3.32	3.88	Pos.
66	A. X. W.		7.19	3.32	3.87	
67	H. A.	Pos.	8.22	4.40	3.82	Neg.
68	L. C.	Pos.	8.31	4.52	3.79	Neg.
69	L. D.	Pos.	7.84	4.11	3.73	Neg.
70	E. W.	Pos.	7.68	3.98	3.70	Neg.
71	R. M.	Neg.	8.19	4.55	3.64	Neg.
72	N. G.	Neg.	8.68	5.08	3.60	Neg.
73	J. S.	Neg.	7.36	3.78	3.58	Neg.
74	J. W.	?	7.76	4.18	3.58	Neg.
75	R. M.	Pos.	6.88	3.32	3.56	Neg.
76	W. W.	Pos.	8.00	4.45	3.55	Neg.
77	R. W.	Neg.	9.04	5.51	3.53	Neg.
78	E. H.	Neg.	8.80	5.31	3.49	Neg.
79	Q. J.	Neg.	7.60	4.12	3.48	Neg.
80	C. L.	Neg.	6.80	3.32	3.48	Undet
81	M. H.	Neg.	8.80	5.36	3.44	Neg.*
82	C. G.	Pos.	7.60	4.18	3.42	Neg.
83	B. P.	Pos.	9.80	6.40	3.40	Neg.*
84	E. W.	Pos.	7.32	3.92	3.40	Neg.*
85	N. E.	Pos.	8.50	5.10	3.40	Neg.
86	J. C.	Pos.	7.76	4.38	3.38	Neg.
87	F. M.	Pos.	6.94	3.65	3.29	Neg.
88	L. M.	?	8.16	4.85	3.31	Neg.
89	H. M.	Pos.	8.00	4.71	3.29	Neg.
90	N. C.	Pos.	7.60	4.38	3.22	Neg.
91	G. F.	Pos.	8.64	5.44	3.20	Neg.
92	D. C.	Pos.	7.28	4.18	3.10	Neg.
93	L. C.	Pos.	7.60	4.52	3.08	Neg.
94	C. G.	Pos.	6.80	3.72	3.08	Neg.
95	B. P.	Pos.	7.20	4.20	3.00	Neg.
96	A. T.	Pos.	6.30	3.50	2.80	Neg.
97	G. M.	Pos.	7.19	4.39	2.80	Neg.
98	M. H.	Neg.	7.50	4.73	2.77	Neg.
99	S. C.	Pos.	6.40	3.65	2.75	Neg.
100	Н. Н.	Neg.	7.20	4.65	2.55	Neg.
101	A. R.	Neg.	7.44	4.91	2.53	Neg.
102	A. H.	Pos.	6.02	3.54	2.48	Neg.
103	N. E.	Pos.	6.11	3.75	2.36	Neg.
104	A. B.	Pos.	7.38	5.04	2.34	Neg.
105	V. D.	Pos.	6.56	4.52	2.04	Neg.

^{*} Takata not determined on the same blood that protein determination was made.

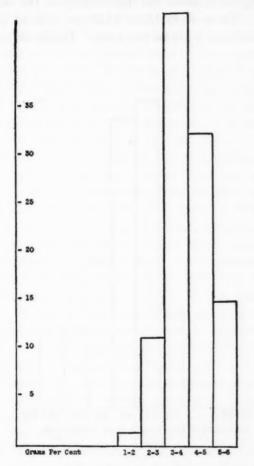
fell in the range above 9 grams per cent. Sixty-seven of the determinations were above the normal range of 6 to 8 grams per cent. If 7.99 is taken as the top normal value, then the majority of the determinations were increased.

The determinations of the albumin fraction present some interesting figures. While the usual situation can be seen to be



that of albumin being lowered in the presence of the higher globulin values, this is not invariably the case. The highest albumin was 6.40 grams per cent, and may represent dehydration, and the lowest albumin was 1.73 grams per cent. It is interesting that the case with this lowest albumin value was the only case in this series that showed any edema as determined by physical

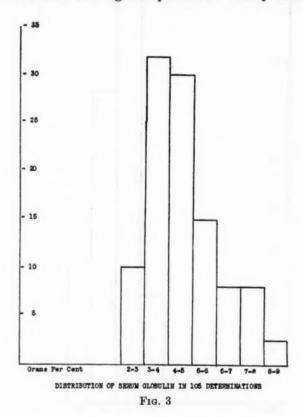
examination. Ninety-three or 88.57 per cent of the values were above 3 grams per cent, and 48 or 45.71 per cent were above 4 grams per cent, which latter figure may be considered as normal



DISTRIBUTION OF SERUM ALBUMIN IN 105 DETERMINATIONS FIG. 2

for the methods used. These figures are interesting as far as the formation of edema is concerned. Calculations of the oncotic pressure of these sera, based on Goverts⁹ figures, show that most of them fall within the normal range. Where the albumin is lowered below the so-called "critical level," the increase in the globulin usually is of such magnitude that it raises the oncotic pressure above the "edema level."

Reference to figure 2 shows the distribution of the determined albumin values. There were 15 or 14.29 per cent of the values that lay between 5 and 6 grams per cent. Thirty-three or 31.43



per cent of the values were between 4 and 5 grams per cent. Forty-five or 42.86 per cent lay between 3 and 4 grams per cent. Eleven or 10.47 per cent lay between 2 and 3 grams per cent. Only one of the determinations lay between 1 and 2 grams per cent.

The most characteristic change in the entire picture is the alteration in the serum globulin fraction. Examination of the

first table and the graphic figures shows this clearly. The highest value is 8.95 grams per cent and the lowest value is 2.04 grams per cent. Eight or 7.62 per cent were between 6 and 7 grams per cent; 15 or 14.27 per cent lay between 5 and 6 grams per cent; 30 or 28.57 per cent of the values were between 4 and 5 grams per cent; 32 or 30.48 per cent were between 3 and 4 grams per cent; and only 10 or 9.52 per cent of the values were between 2 and 3 grams per cent. It can be seen that 95 or 90.4 per cent of the globulin values were abnormally high (above 3 grams per cent). Of the 73 total protein determinations whose values fell within the range of 6 to 9 grams per cent, 63 or 86.3 per cent had globulins that were above 3 grams per cent.

We were particularly interested in correlating the changes in the serum protein with the clinical condition of the patient. There was no definite correlation of the proteins with the pathological condition that was present. In other words, the finding of a rectal stricture or a massive granulomatous lesion of the vulva was not necessarily associated with hyperglobulinemia. However, in those cases where there was considerable concurrent pyogenic infection, the globulins were quite likely to be increased. In the acute cases, an early increase in the globulins was encountered. In two of the cases it was as early as three weeks after the appearance of the initial lesion. We have felt that the activity of the disease, and not the pathological picture present, is the factor that is associated with the increase in the globulins.

In 17 patients, two or more determinations of the plasma proteins have been made at varying intervals. We found that the values of the globulins were prone to show rapid and significant variations over rather short periods of time. Table 2 shows these variations. The decrease of the globulins was usually associated with improvement of the clinical condition of the patient.

In conjunction with the determination of the plasma proteins. the Takata-Ara reaction was determined in ninety-nine instances. These determinations were made on the same serum sample that was used to determine the proteins, save in those where the exception is indicated. The Takata-Ara reaction was found to be positive in fifty instances and negative in forty-nine. In the

TABLE 2
THE VARIATION OF THE PLASMA PROTEINS IN THOSE PATIENTS ON WHOM MORE THAN ONE DETERMINATION HAS BEEN MADE

	NAME	DATE	WASSER- MANN	TOTAL PROTEIN	ALBUMIN	GLOBULIN	TAKATA
	1 m	3/31/36	Pos.	13.30	4.50	8.80	Pos.
1	A. T. {	4/18/36	Pos.	6.30	3.50	2.80	Neg.
2	N. W. {	3/20/37	Neg.	11.20	2.25	8.95	Pos.
2	N. W.	5/29/37	Neg.	10.40	3.32	7.08	Pos.
	1	2/18/38	Pos.	10.80	3.65	7.15	Pos.
3	N. E.	2/24/38	Pos.	8.50	5.10	3.40	Neg.
		2/28/38	Pos.	6.11	3.75	2.36	Neg.
4	C. L. {	3/10/37	Neg.	8.96	3.98	4.98	Pos.
4	C. L.	2/ 4/38	Neg.	11.20	4.65	6.55	Pos.
	(3/ 1/37	Neg.	9.20	3.98	5.22	Pos.
5	I. P. {	4/17/37	Neg.	8.00	3.42	4.58	Pos.
		2/12/38	Neg.	7.92	3.78	4.14	Pos.
	. (1/26/38	Pos.	9.52	4.32	5.20	Pos.
		1/12/38	Pos.	6.59	2.15	4.44	Pos.
6	L. D. {	1/ 4/38	Pos.	7.84	4.11	3.73	Neg.
		2/17/38	Pos.	9.84	3.92	5.92	Unde
		2/25/38	Pos.	6.60	2.15	4.45	Pos.
	1	4/22/37	Neg.	8.88	2.59	6.29	Pos.
		4/27/37	Neg.	10.24	3.19	7.05	Pos.
7	P. W. {	2/21/38	Pos.	10.66	3.05	7.61	Pos.
		3/10/38	Pos.	10.32	2.92	7.40	Pos.
	1		Pos.	10.40	2.92	7.48	Pos.
		4/ 9/37	Pos.	8.00	2.92	5.07	Pos.
8	K. D.	4/10/37	Pos.	7.76	3.32	4.44	Unde
		4/15/37	Pos.	8.24	3.25	4.99	Pos.
9	J. M. {	1/20/37	Pos.	8.80	3.98	4.82	Pos.
8	J. M.	2/12/38	Pos.	8.00	3.98	4.02	Pos.
10	G. W. {	8/20/37	Pos.	7.60	2.99	4.61	Pos.
10	G. W.	10/20/37	Pos.	6.80	2.79	4.01	Pos.
		2/11/38	Neg.	9.60	5.31	4.29	Neg.
11	M. H.	2/14/38	Neg.	8.80	5.36	3.44	Neg.
		2/22/38	Neg.	7.50	4.73	2.77	Neg.
12	w. w. {	2/15/38	Pos.	9.36	5.24	4.12	Pos.*
14	W. W.	2/22/38	Pos.	8.00	4.45	3.55	Neg.

TABLE 2-Concluded

	NAME	DATE	WASSER- MANN	TOTAL PROTEIN	ALBUMIN	GLOBULIN	TAKATA
13	L. C. {	2/20/38 2/24/38	Pos. Pos.	8.31 7.60	4.52 4.52	3.79 3.08	Neg. Neg.
14	В. Р. {	1/23/38 2/ 7/38	Pos. Pos.	7.20 9.80	4.20 6.40	3.00 3.40	Neg.*
15	E. W. {	5/21/37 6/ 3/37	Pos. Pos.	7.32 7.68	3.92 3.98	3.40 3.70	Neg.*
16	E. R. {		Pos. Pos.	11.20 9.40	3.65 2.52	7.55 6.92	Pos. Pos.
17	м. с. {		Pos.	8.80 8.20	3.05 3.05	5.75 5.15	Pos.

^{*} Takata not determined on the same blood that protein determination was made.

fifty positives, the lowest globulin was 3.88 grams per cent. Of these positive reactions, forty-seven were associated with a reversal of the albumin and globulin ratio. Three of the positive reactions were not associated with reversal of the ratio. These were associated with either very high globulin values or the determined values were quite close to each other and the ratio was almost exactly 1:1. On the other hand, six negative reactions were associated with reversal of the ratios. In some of these latter cases, the Takata-Ara was not determined on the same sample upon which the protein determination was made. In view of the rapid and significant changes which may occur in the globulin values the variable reactions may be accounted for. These various reactions are presented in table 1.

The Takata Reaction was introduced by Takata³⁸ in 1925 as a test to differentiate lobar and bronchopneumonia and later applied by Takata and Ara³⁹ to the detection of changes in spinal fluid. Jezler¹⁶ applied the test to the diagnosis of liver disease in 1930. Jeghers¹⁴ suggested the Takata-Ara reaction as a means of detecting hyperglobulinemia in multiple myeloma in 1937, and in a note reported the detection of hyperglobulinemia in a

case of lymphogranuloma venereum. Numerous investigators have discussed its diagnostic significance in the study of liver disease and have commented upon the limitations of the test. Nevertheless, it has enjoyed a somewhat quasi-pathognomonic reputation insofar as cirrhosis of the liver is concerned. as the significance of the reaction is doubtful, so also is the actual mechanism of the production of a positive reaction. Excellent reviews of the subject of the mechanism and clinical significance are found in the papers Kirk¹⁹, and Chasnoff and Solomon^{3, 4}. Takata thought that the precipitate which constitutes the positive reaction was mercuric oxy-chloride. The protective albumins in normal sera prevented the formation of the precipitate, but when the globulins become excessive then the reagents precipitate. Jezler and others have upheld this contention. Kirk sought to relate the reaction to absolute changes in the serum globulins. Others have felt that increase in the euglobulins is the factor responsible for the positive reaction. Other hypotheses have been proposed that have to do with changes in fatty acids, increase in ammonia, and alterations of the salt content. Gros¹⁰ has analyzed the precipitate and has found that it was 80 per cent organic material, and states that the precipitate is mostly albumin. In a few analyses of the nitrogen in the precipitate, we have found that the nitrogen-containing precipitate does not correspond quantitatively with any of the various fractions of globulin that are precipitated by 15, 18, or 21 per cent sodium sulphite.

In view of the noted alterations of the serum proteins in liver disease and the association of the positive Takata-Ara reaction with certain forms of such hepatic pathology, we studied the liver functions of 27 patients in conjunction with protein and Takata reaction. The other tests employed were the Van den Bergh reaction, Bromsulphthalein test, urobilinogen determination, and galactose tolerance test. It is unlikely that significant diffuse hepato-cellular pathology exists without alteration in some one or more of these tests. The results of these determinations are presented in table 3.

In 2 of the patients, H. W. and L. T., there is no explanation

of the small amount of dye retention. Neither of these cases had a positive Takata-Ara reaction and none of the other tests were positive. L. T. was obviously in a severe state of heart

TABLE 3 TABLE OF PLASMA PROTEINS, TAKATA-ARA REACTIONS AND LIVER FUNCTION TESTS

	NAME	WASS.	T.P.	ALB.	GLOB.	TAKATA	BROM,	V AND B	GALACT.	UROB
							per cent			
1	N. W.	Neg.	11.2	2.25	8.95	Pos.	0	0.2	1.62	1/10
2	A. T.	Pos.	13.3	4.50	8.80	Pos.	0	0.2	1.96	1/10
3	P. W.	Pos.	10.4	2.92	7.48	Pos.	0	0.2	1.96	
4	N. E.	Pos.	10.8	3.65	7.15	Pos.	0	1.0	2.05	1/20
5	A. B.	Neg.	10.48	3.85	6.63	Pos.	0	0.2	0.365	1/10
6	C. L.	Neg.	11.2	4.65	6.55	Pos.	0	0.2	0.496	1/20
7	E. E.	Pos.	11.2	5.64	5.56	Pos.*	0	0.2		1/10
8	L. J.	Pos.	9.04	3.85	5.19	Pos.	0	0.2		1/20
9	R. H.	Pos.	10.4	5.04	5.40	Pos.*	0	0.2	0.730	1/10
10	F. S.	Neg.	6.96	1.73	5.23	Pos.	0	0.2	0.696	1/10
11	I. P.	Neg.	9.20	3.98	5.22	Pos.	0	0.3	0.628	1/10
12	H. W.	Neg.	8.40	3.32	5.08	Pos.	10	0.2	0.656	1/10
13	K. D.	Pos.	8.00	2.93	5.07	Pos.	0	0.2		1/10
14	B. M.	Pos.	8.16	3.32	4.84	Pos.	0	0.2		1/10
15	J. B.	Pos.	8.80	3.98	4.82	Pos.	0	0.2	0.402	1/10
16	E. J.	Pos.	8.00	3.32	4.68	Pos.	0	0.2		1/10
17	G. W.	Pos.	7.60	2.99	4.61	Pos.	0	0.2		1/10
18	L. T.	Pos.	9.20	4.64	4.56	Neg.	5	0.2	1.380	1/10
19	M. H.	Neg.	9.60	5.31	4.29	Neg.	0	0.2	1.080	1/10
20	L. T.	Neg.	8.24	3.98	4.26	Neg.*	18	0.2		1/10
21	W. W.	Pos.	9.36	5.24	4.12	Pos.*	0	0.2		1/10
22	B. T.	Pos.	7.20	3.32	3.88	Pos.	0	0.2		1/10
23	E. H.	Neg.	8.80	5.31	3.49	Neg.	0	0.2	1.28	1/10
24	R. W.	Neg.	9.40	5.51	3.49	Neg.	0	0.2	0.084	1/10
25	S. C.	Pos.	6.40	3.65	2.75	Neg.	0	0.2	0.796	1/10
26	A. R.	Neg.	7.44	4.91	2.53	Neg.	0	0.2	0.670	1/10
27	V. D.	Pos.	6.56	4.52	2.04	Neg.	0	0.2		1/10

^{*} Takata not determined on the same blood that protein determination was made.

failure at the time of the examination. She deserted the hospital before studies were completed.

Only one of the cases showed an increase in the serum bilirubin as determined by the Van den Bergh reaction. This case, N. E.,

was a case of pernicious anemia. All the galactose tolerance tests and all the urobilinogen determinations fell within normal limits. In fact, none of the positive Takata reactions where studied showed any corroborative evidence of liver disease in the form of positivity of other tests of liver function. The significant fact seems to be that the test is positive in the presence of reversal of the albumin and globulin ratios. This is not incompatible with the finding of negative reactions in nephrosis and those cases where the globulins are not increased. Table 2 shows variations in the Takata-Ara reaction, parallel with changes in the concentration of the globulins. It will be noted that as the globulins decrease in concentration to where they constitute

TABLE 4

	NAME	T.P.	ALB,	GLOB.	EUG.	PsGI	PsGII	FIB.	TAKATA
1	J. K.	6.31	2.38	3.93	1.71	1.48	.74	. 53	Pos.
2	A. H.	6.03	3.55	2.48	.60	1.41	.47	. 63	Neg.
3	N. E.	6.12	3.76	2.36	1.06	.77	. 53	. 55	Neg.
4	L. C.	8.31	4.52	3.79	2.04	1.19	.56	.64	Neg.
5	P. W.	10.66	3.05	7.61	4.36	2.58	. 67	.93	Pos.
6	L. D.	6.60	2.15	4.45	3.10	.44	.91	.98	Pos.
7	H. A.	8.22	4.40	3.82	1.44	1.68	.70		Neg.
8	H. W.	8.39	4.21	4.18	2.13	1.32	.73		Pos.
9	М. Н.	7.50	4.74	2.77					Neg.
10	G. M.	7.19	4.39	2.80	1.10	1.08	.62		Neg.

less than 50 per cent of the total protein concentration, the Takata-Ara reaction becomes negative in the same individual where it previously had been positive. Such a relationship between changes in globulin were noted also by Gros in 1937.

Table 4 presents a few sera in which the euglobulin and the pseudo-globulin fractions have been determined in an attempt to correlate the Takata-Ara reaction with increase in euglobulin. This confirms the finding of Gutman, Gutman, Jilson and Williams¹² that the increase in the globulin fraction is due to an increase in the euglobulin fraction. In most of these determinations, the euglobulins are increased to a concentration greater than their usual 15 to 20 per cent of the total globulin. Yet, in spite of one or two fairly marked increases, the Takata-Ara

reaction is negative and is associated with the normal relationship of the A/G ratio. The positive reactions in this group are associated with reversals of the ratio, and in the group, the major part of the increase in the globulin is due to the increment in the euglobulin fraction.

The formol-gel test has been used to detect hyperglobulinemia in the Leishmaniases^{24, 7}, and in some of these instances, the test assumed a quasi-pathognomicity. Wise and Gutman⁴² have applied this reaction to detect hyperglobulinemia in lymphogranuloma venereum and other diseases. Bing² gives an excellent review and bibliography of this phase. Similarly, other globulin reactions have been utilized as diagnostic procedure in schistosomiasis (Sia³⁵), and the increased euglobulin has been considered the responsible globulin fraction. Although no systematic investigation has been undertaken of this point, we have found a few positive reactions in cases which have been known to have marked increase in their serum globulin.

COMMENT

In general in this group of patients, 30.5 per cent showed determinations of total proteins of 9 grams per cent; the average being 8.55 grams per cent, which can be considered in moderate excess of the usually quoted 6 to 8 grams per cent.

In 90.4 per cent of these cases, the globulin fraction was increased; the average in this instance being 4.56 grams per cent. 44.29 grams per cent of the albumin determination lay below 4 grams per cent. The average was 3.99 grams per cent.

It is evident that the increase in the total protein is due to an elevation of the globulin fractions. Because of the general impression that syphilis is associated with hyperglobulinemia, it might be inferred that this is the operative factor in this material, since 70 per cent of these cases show a positive Wassermann reaction. However, the infrequency of hyperglobulinemia in syphilis has been amply substantiated by Bing¹ in an analyses of 3,695 cases, and again by Jersild¹⁵ in 10,043 cases. Rosen, Rosenfeld and Lyons³² have not found uncomplicated syphilis to be associated with an increase in the plasma proteins. We have not found marked increases in the globulin fraction of syphilis

except in those individuals who also show a positive Frei reaction. It will be noted that some of the highest globulin values are found in individuals who have repeatedly shown negative Wassermann reactions.

In 54 of the determinations, there was a reversal of the albumin-globulin ratio. Such occurrence was in most instances associated with an absolute increase in serum globulin with a small and more infrequently marked decrease in the serum globulin. The factors operative in the production of the hypoalbuminemia are probably the same as suggested by Peters and Eisenman²⁷ in connection with other disease.

SUMMARY AND CONCLUSION

1. The material presented represents a study of 79 patients with lymphogranuloma venereum on whom a total of 105 determinations of serum protein fractions were made.

2. An unusually high incidence of positive Takata-Ara reactions was obtained. These seem to parallel reversals in the

albumin-globulin ratio.

3. The globulins, which comprise the major increase of the protein fractions, show marked variations in short periods of time. Where the Takata-Ara test was positive in the presence of hyperglobulinemia, marked lowering of the globulin resulted in normal A/G ratios with negative Takata-Ara reactions.

4. In the cases studied, there was no evidence of liver damage as ascertained clinically and by use of the galactose tolerance, bromosulphthalein excretion test, urobilingen, and serum bilirubin determinations.

5. Brief mention is made of other related globulin tests.

6. Changes in globulin level would seem to parallel changes in activity of the disease.

7. The persistent hyperglobulinemia is evidence that the disease is chronically active.

8. 90.4 per cent of these patients show a hyperglobulinemia which may be of diagnostic and prognostic significance.

Grateful acknowledgment is made to Dr. Collier F. Martin for much assistance with the present study.

THE INCIDENCE OF INTESTINAL PARASITES*

An Analysis of 2,265 Routine, Consecutive Stool Examinations in the Outpatient Dispensaries of Charity Hospital of Louisiana at New Orleans

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An increasing realization of the practical importance of intestinal parasites, and particularly of undiagnosed parasitic infection, has led to the compilation of numerous reports from various sections of the United States as well as from foreign countries. Among these may be listed as typical:

A study by Wenrich, Stabler and Arnett¹ of 401 first-year college students in Philadelphia, with 176 positive findings.

A study by Fantham and Porter² of 563 hospital and private patients in Montreal, with 208 positive findings.

A study by Andrews³ of 2,302 native and foreign subjects in Fresnillo, Mexico, with 1,770 positive findings.

A study by Adams and Webb⁴ of 428 prison and reform school subjects in Mauritius, South Africa, with 99 positive findings.

A study by Byrd⁵ of 537 individuals on relief in Athens, Georgia, with 239 positive findings.

A study by Bradley and Johnson⁶ of 170 children in Tulsa, Oklahoma, and the vicinity, with 23 positive findings.

A study by Swartzwelder⁷ of 291 medical students in New Orleans, with 85 positive findings.

Even this casually selected list of recent surveys will make certain points clear. In the first place, most of them concern themselves with special groups of subjects and therefore exhibit a more or less high degree of selectivity. In the second place,

^{*}Received for publication December 12, 1938.

a more detailed analysis reveals that some of them concern themselves only with the search for a special parasite or species of parasite, and not with parasitic infection in general. In the third place, and most important, all of these surveys exhibit an astonishingly high degree of positive findings, a degree which becomes the more extraordinary when it is recollected that not a single one of them is concerned exclusively with subjects in whom parasitic infection was suspected. Some such subjects are included in each group, it is true, but their inclusion is accidental, and the number is small, both actually and relatively. The practical importance of this very large number of undiagnosed parasitic carriers from the standpoint of public health and sanitation is so obvious as to need no discussion.

Our own contribution is made for two reasons. In the first place, the subject is a very important one, as we have just pointed out. In the second place, so far as we are aware, no report from this locality or from other localities is entirely unselective. Faust and Headlee's study* of 4,270 white clinic patients in New Orleans comes nearest to that ideal, but it does not include negro patients. It has therefore seemed to us worth while to put on record the results of a large series of consecutive, routine stool examinations on entirely unselected subjects, without any attempt to draw conclusions therefrom, or to evaluate the infections with respect to pathogenicity.

The data for this report comprise 2,265 consecutive stool examinations made as part of the ordinary routine in the outpatient dispensaries of Charity Hospital in New Orleans. A small number of suspected pathologic subjects are included, but for the most part the stool examination was requested as one phase of a routine study and not because the patient presented symptoms referable to the gastrointestinal tract or suggestive of parasitic infection. The subjects are entirely unselected and include white and negro patients and male and female patients of all age groups. As a matter of interest, it might be stated that the white and negro admissions to the out-patient department of Charity Hospital are practically the same, and that the male and female admissions differ only slightly. The statistics on the new patients for the hospital year ending June 30, 1938, show: 8,700 white male and 8,005 colored male patients. 8,720 white female and 9,238 colored female patients, 17,420 white and 17,303 colored patients (total), 16,765 male and 17,958 female patients (total). The chief disparity, it will be noted, is between the white and negro female patients.

The percentage of positive findings reaches the very high figure of 40.574 per cent, and the actual figure, if it could be correctly arrived at, would probably be even higher. Several sources of error immediately suggest themselves:

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1. The necessarily brief time spent in the examination of each specimen.

2. The limited (usually single) examinations of each stool, and the limited methods of examination of each stool.

3. The personal equation, which, as always, is a definite factor. Some comment on these sources of error is justified. In the first place, stool examinations, even when they average only 10 or 12 a day, constitute merely one item of routine work in a busy laboratory, in which hundreds of patients are handled and hundreds of examinations are made each day, so that an undue amount of time cannot be spent on any one phase of the work. In the second place, repeated examinations are usually impossible, even when they are desirable, because of lack of cooperation on the part of the patients, the majority of whom fall into the lower levels of intelligence.

As to the preparation and examination of the specimens, a routine has been established whereby a considerable degree of efficiency has been obtained with a minimum expenditure of time and effort. The method of "concentration by centrifugalization," as outlined by the author, has been used uniformly because usually only a single method of examination can be employed for each specimen. When cysts are present, iodine solution is added to the preparation, and a cover glass is applied to permit examination and identification under high, dry magnification. No examination of the unconcentrated specimen is made for trophozoites of the protozoa, nor is any special examination made for Enterobius vermicularis (ova, larvae, or adult worms); the latter omission, however, is not regarded as serious because positive findings are infrequent in passed stools.

The tables are presented without attempt at analysis, for the material is intended chiefly for the clinical pathologist and for the clinician, who will probably prefer to draw their own conclusions from the absolute results. One or two general statements, however, may be of interest.

The material, as we have said, includes 2,265 stool examinations, 919 of which, 40.574 per cent, were positive. Sixteen species of parasites were identified, including, in the order of frequency: Trichocephalus trichiurus, Endamoeba coli, Giardia lamblia, Ascaris lumbricoides, Endamoeba histolytica, Strongyloides stercoralis, Endolimax nana, Enterobius vermicularis, Necator americanus, Taenia saginata (spp.), Heterodera radi-

TABLE 1
Infection with One Species of Parasite

	NUMBER	RELATIVE PER CENT	ABSOLUTE PER CENT	TOTAL APPEAR- ANCE	RELATIVE PER CENT	ABSOLUTE PER CENT
Trichocephalus trichiurus	145	15.766	6.401	295	32.100	13.024
Endamoeba coli	157	15.995	6.931	281	30.576	12.409
Giardia lamblia	120	13.057	5.298	182	15.560	8.035
Ascaris lumbricoides	63	6.855	2.781	146	14.798	6.455
Endamoeba histolytica	42	4.570	1.854	77	8.366	3.399
Strongyloides stercoralis	38	4.124	1.677	47	5.114	2.074
Endolimax nana	28	3.046	1.231	53	5.756	2.339
Enterobius vermicularis	27	2.936	1.192	45	4.896	1.986
Necator americanus	25	2.720	1.103	38	4.124	1.677
Taenia saginata (spp.)	9	0.978	0.039	10	1.088	0.043
Heterodera radicicola	6	0.652	0.026	6	0.652	0.026
Chilomastix mesnili	4	0.435	0.017	6	0.652	0.026
Iodamoeba butschlii	2	0.217	0.008	9	0.978	0.039
Trichomonas hominis	2	0.217	0.008	2	0.217	0.008
Hymenolepis nana	1	0.108	0.004	1	0.108	0.004
Mites (Tyroglyphus spp.)	1	0.108	0.004	2	0.217	0.008
Cyst (species undeter- mined)	10	1.088	0.043	12	1.305	0.529
Totals	680	73.933	30.507			

cicola, Chilomastix mesnili, Iodamoeba butschlii, Hymenolepis nana, Mite (Tyroglyphus spp.).

Details of the incidence of these various parasites are shown in tables 1 through 6, and again certain general statements may be made:

- 1. When the infection was with a single species, Protozoa (365) appeared more frequently than Helminthes (314).
- 2. When the infection was multiple (239), the situation was reversed, and Helminths (76) appeared more frequently than

Protozoa (52). On the other hand, multiple infections were more frequently mixed than otherwise.

TABLE 2
Infection with Two Species of Parasites

	NUM- BER	BELATIVE PER CENT	ABSOLUTE PER CENT
Trichocephalus trichiurus and Ascaris lumbricoides	40	4.352	1.766
Trichocephalus trichiurus and Endamoeba coli	38	4.124	1.677
Trichocephalus trichiurus and Giardia lamblia Trichocephalus trichiurus and Endamoeba his-	14	1.522	0.618
tolyticaTrichocephalus trichiurus and Enterobius vermicu-	5	0.543	0.220
laris	4	0.435	0.017
Trichocephalus trichiurus and Necator americanus	3	0.326	0.132
Trichocephalus trichiurus and Hymenolepis nana Trichocephalus trichiurus and Strongyloides ster-	3	0.326	0.132
coralis	2	0.217	0.008
termined)	2	0.217	0.008
Endamoeba coli and Giardia lamblia	15	1.632	0.662
Endamoeba coli and Ascaris lumbricoides	9	0.978	0.039
Endamoeba coli and Endamoeba histolytica	10	1.088	0.043
Endamoeba coli and Hymenolepis nana	8	0.870	0.352
Endamoeba coli and Enterobius vermicularis	5	0.543	0.220
Endamoeba coli and Necator americanus	3	0.326	0.132
Endamoeba coli and Strongyloides stercoralis	3	0.326	0.132
Endamoeba coli and Iodamoeba butschlii	1	0.108	0.004
Endamoeba coli and Chilomastix mesnili	1	0.108	0.004
Giardia lamblia and Ascaris lumbricoides	6	0.652	0.026
Giardia lamblia and Hymenolepis nana	6	0.652	0.026
Giardia lamblia and Endamoeba histolytica	2	0.217	0.008
Giardia lamblia and Necator americanus	1	0.108	0.004
Giardia lamblia and Strongyloides stercoralis	1	0.108	0.004
Giardia lamblia and Enterobius vermicularis	1	0.108	0.004
Enterobius vermicularis and Ascaris lumbricoides	2	0.217	0.008
Enterobius vermicularis and Necator americanus	1	0.108	0.004
Endamoeba histolytica and Ascaris lumbricoides	1	0.108	0.004
Endamoeba histolytica and Iodamoeba butschlii Enterobius vermicularis and Strongyloides ster-	1	0.108	0.004
coralis	1	0.108	0.004
Total	189		

^{3.} All the infections with 4 species (7) were mixed Helminthes and Protozoa.

TABLE 3
INFECTION WITH THREE SPECIES OF PARASITES

Trichocephalus trichiurus and Endamoeba coli and Ascaris lumbricoides Trichocephalus trichiurus and Endamoeba coli and Giardia lamblia Trichocephalus trichiurus and Endamoeba coli and Necator americanus Trichocephalus trichiurus and Endamoeba coli and Endamoeba histolytica Trichocephalus trichiurus and Ascaris lumbricoides and Giardia lamblia Trichocephalus trichiurus and Ascaris lumbricoides and Endamoeba histolytica Trichocephalus trichiurus and Ascaris lumbricoides and Strongyloides stercoralis Trichocephalus trichiurus and Ascaris lumbricoides and Necator americanus Trichocephalus trichiurus and Ascaris lumbricoides and Necator americanus Trichocephalus trichiurus and Ascaris lumbricoides and Taenia saginata (spp) Trichocephalus trichiurus and Ascaris lumbricoides and Iodamoeba butschlii Trichocephalus trichiurus and Endolimax nana and Enterobius vermicularis Trichocephalus trichiurus and Endolimax nana and mites Endamoeba coli and Giardia lamblia and Endamoeba histolytica Endamoeba coli and Necator americanus and Endamoeba histolytica Endamoeba coli and Necator americanus and Endamoeba histolytica Endamoeba coli and Riardia lamblia and Endolimax nana Giardia lamblia and Endamoeba histolytica and Iodamoeba butschlii Giardia lamblia and Endamoeba histolytica and Iodamoeba butschlii Giardia lamblia and Endolimax nana and Iodamoeba butschlii Endolimax nana and Endamoeba histolytica and Iodamoeba butschlii Endolimax nana and Endamoeba histolytica and Iodamoeba butschlii Endolimax nana and Endamoeba histolytica and Iodamoeba butschlii.	NUM- BER	RELATIVE PER CENT	ABSOLUTE PER CENT
Ascaris lumbricoides Trichocephalus trichiurus and Endamoeba coli and Giardia lamblia Trichocephalus trichiurus and Endamoeba coli and Necator americanus Trichocephalus trichiurus and Endamoeba coli and Endamoeba histolytica Trichocephalus trichiurus and Ascaris lumbricoides and Giardia lamblia Trichocephalus trichiurus and Ascaris lumbricoides and Endamoeba histolytica Trichocephalus trichiurus and Ascaris lumbricoides and Strongyloides stercoralis Trichocephalus trichiurus and Ascaris lumbricoides and Necator americanus Trichocephalus trichiurus and Ascaris lumbricoides and Taenia saginata (spp) Trichocephalus trichiurus and Ascaris lumbricoides and Iodamoeba butschlii Trichocephalus trichiurus and Endolimax nana and Enterobius vermicularis Trichocephalus trichiurus and Endolimax nana and mites Endamoeba coli and Giardia lamblia and Endamoeba histolytica Endamoeba coli and Necator americanus and Endamoeba histolytica Endamoeba coli and Necator americanus and Endamoeba histolytica Endamoeba coli and Necator americanus and Enterobius vermicularis Endamoeba coli and Giardia lamblia and Endolimax nana Giardia lamblia and Endamoeba histolytica and Iodamoeba butschlii Giardia lamblia and Endolimax nana and Iodamoeba butschlii			
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Iodamoeba butschlii	1	0.108	0.004
butschlii	1	0.108	0.004
Endolimer nane and Endamoche histolytica and	1	0.108	0.004
Iodamoeba butschlii	1	0.108	0.004
Endolimax nana and Endamoeba histolytica and Chilomastix mesnili	1	0.108	0.004
Endolimax nana and Endamoeba histolytica and			
Enterobius vermicularis	43	0.108	0.004

Analysis of the data by race, sex, and age appears in tables 7 and 8. No special comment is needed except as to the incidence

TABLE 4
INFECTION WITH FOUR SPECIES OF PARASITES

	NUM- BER	RELATIVE PER CENT	ABSOLUTE PER CENT
Trichocephalus trichiurus and Endamoeba coli and Ascaris lumbricoides and Endamoeba histolytica	2	0.217	0.008
Trichocephalus trichiurus and Endamoeba coli and Ascaris lumbricoides and Iodamoeba butschlii	1	0.108	0.004
Trichocephalus trichiurus and Endamoeba coli and Ascaris lumbricoides and Strongyloides stercoralis	1	0.108	0.004
Trichocephalus trichiurus and Endamoeba coli and Giardia lamblia and Endamoeba histolytica Trichocephalus trichiurus and Giardia lamblia and	2	0.217	0.008
Ascaris lumbricoides and Enterobius vermicularis	1	0.108	0.004
Total	7		

TABLE 5
SUMMARY OF INFECTIONS IN RELATION TO NUMBER OF PARASITES

	NUMBER	PER CENT	ABSOLUTE PER CENT
Infection with one species	680	73.916	30.022
Infection with two species		20.576	8.344
Infection with three species		4.679	1.898
Infection with four species		0.076	0.030

TABLE 6
SUMMARY OF INFECTIONS IN RELATION TO PARASITES

	NUMBER	RELATIVE PER CENT	ABSOLUTE PER CENT
Infections with Helminths only	390	42.437	17.218
Infections with Protozoa only	417	45.375	18.410
minths	111	12.078	4.909
Infections with mites only	1	0.108	0.004
Total	919		

of Ascaris (table 9), which is very striking. In 146 cases, the infection was with Ascaris only in 63 patients, 58 of whom were

under 16 years of age. Of the 58 patients infected with Ascaris and one other species, 56 were under that age. Of the 20 pa-

TABLE 7
RACE AND SEX DISTRIBUTION

Total positives	910	(40 57497
White males.		
White females		
Colored males		
Colored males		
Colored females	101	(17.000%)
Infection with Helminth only:		
White males	123	
White females	139	
Colored males	49	
Colored females	59	
Total white	262	
Total colored	108	
Total males	172	
Total females	198	
Infection with Protozoa only:		
White males	148	
White females.		
Colored males		
Colored females		
Total white	192	
Total colored	124	
Total males	195	
Total females	221	
Infection with Helminths and Protozoa:		
White males	40	
White females	36	
Colored males	17	
Colored females	16	
Total white	76	
Total colored	33	
Total males	57	
Total females	52	

tients infected with Ascaris and 2 other species, 19 were under that age. Of the 5 patients infected with Ascaris and 3 other

TABLE 8

AGE	NUMBER	PERC	ENTAGE
1-5	225	24.483	
6–10	327	35.582	
11-15	133	14.472	
16-20	45	4.897	
Total	730	79.434	under 20
21-25	28	3.046	
26-30	45	4.897	
31-35	36	3.916	
36-40	34	3.699	
Total	143	15.558	between 20 and 40
41-45	18	1.958	
46-50	9	0.978	
51-60	14	1.522	
61-70	3	0.326	
71-80	1	0.108	
81 and over	1	0.108	
Total	46	4.998	over 40

TABLE 9
INFECTION WITH ASCARIS LUMBRICOIDES

1. Age distribution:		
Ascaris only	63-Under 16 years	s 58
Ascaris and one other	58-Under 16 years	s 56
Ascaris and two others	20-Under 16 years	s 19
Ascaris and three others	5-Under 16 vear	g 5
Total	146	138
2. Race and sex distribution:		
White males		28
White females		. 47
		75
Colored males		37
Colored females		. 34
		71

species, all were under that age. In short, in the 146 cases of this variety of infection, 138 occurred in patients under 16 years of

age. Twenty-eight of the patients were white males, and 47 white females, a total of 75 white patients. Thirty-seven were colored males and 34 colored females, a total of 71 colored patients. The sex and race incidence is practically parallel, but there is an overwhelming preponderance of this variety of infection in the younger age group.

From the standpoint of infection by individual parasites the following general statements may be made:

Trichocephalus trichiurus. This is the most frequent Helminth encountered, either alone (145 of 680 cases) or in mixed infections (295 cases). In 189 double infections it was found with Helminths alone 49 times and with Protozoa alone only 62 times. In 38 of these double infections it was associated with E. coli. It appeared in 30 of 43 triple infections, in 11 of which it was associated with E. coli, and in all 7 quadruple infections in the series.

Endamoeba coli. This is the most frequent Protozoa encountered, either alone (157) or in mixed infections (281). It was present in 93 of 189 double infections, in which it appeared with Protozoa only 35 times, and with Helminths only 58 times. It was also present in 17 of 43 triple infections and in 6 of 7 quadruple infections.

Giardia lamblia. This parasite was present in 182 cases, in 120 of them as a single infection. It was present in 46 double infections, 13 triple infections, and 3 quadruple infections. It was associated with Helminths 24 times, with other Protozoa 23 times, and with mixed infections 15 times.

Ascaris lumbricoides. This parasite (table 9) appeared alone 63 times and in association 80 times. It appeared in 40 cases with Trichocephalus only, in 45 with Helminths only, in 16 with Protozoa only, and in 22 with mixed Helminths and Protozoa. The peculiarities of age incidence have already been commented upon.

Endamoeba histolytica. The incidence of 77 (3.399 per cent absolute) is based only on the finding of typical cysts and is therefore somewhat lower than the figures usually achieved, which take into consideration trophozoites and precystic forms. It is our opinion that a considerable number of cysts of undetermined species could properly have been classed as E. histolytica, but it is not our policy to report as positive any doubtful or unconfirmed findings, and they are therefore not included.

Strongyloides stercoralis. This parasite appears more frequently alone (38) than in association with other parasites (9). The age, race, and sex incidence is somewhat unusual. It appeared in 28 males against 19 females, and in 19 subjects under 20 years of age against 28 over that age. It appeared only 3 times in negro subjects, all of whom were males. In the 44 white subjects there were 25 males and 19 females.

Enterobius vermicularis. This parasite appeared 27 times alone and 18 times in association with other parasites. Of the patients infected 18 were males and 27 females; 39 were under 20 and 6 over 20 years of age; 41 were white, of whom 17 were male and 24 female, and only one of the 4 colored patients was a male.

Necator americanus. A casual check of the data reveals that the majority of subjects studied belong to the urban poulation. Most of the hookworm infections were found in the small number of patients from rural districts.

Endolimax nana. This parasite appeared 53 times. Its chief importance lies in the fact that it may be confused with E. histolytica.

Taenia saginata. Of the 10 infections with this parasite, only one was in association with other parasites (Trichocephalus and Ascaris in a triple infection, in a white female under 15 years of age). Eight of the remaining infections were also in female patients, and colored males were not represented in the series. Only two of the 10 subjects were over 20 years of age.

The other parasites appeared too infrequently to warrant a detailed analysis.

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THE LABORATORY DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS*

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Further experience with a considerable number of sporadic cases of infectious mononucleosis has led the authors to continue to believe, as they did in 1935, that only a small portion of the actual number of cases which occur are properly diagnosed. A fair proportion of our cases and of those reported by others were first diagnosed as being infectious mononucleosis only because of a routine hospital blood count. Probably many cases not subjected to hematologic study are clinically diagnosed as influenza, sore throat, Vincent's angina or lymphadenitis.

The clinical features of the disease are admirably discussed by Sprunt^{19,20}, and McKinlay, Downey and Stasney¹⁵ in American literature, by Tidy²⁴ in England, and by Lehndorff and Schwarz¹² on the Continent. Very little can be added to these splendid articles. Clinically the cases seen by us have shown most of the variations described by the above authors and most of them have been quite like the typical case described by Sprunt²⁰ as follows:

A young man begins to feel badly with an indefinite malaise, a slight headache and perhaps pain in the back or in the abdomen. He loses his appetite, is lack-adaiscal, and must drive himself in accomplishing his usual routine. During the next two or three days he grows worse instead of better, he may have chill and sweats, his discomfort increases, and he applies for medical advice. The temperature is found to be elevated (103° to 105°), the cheeks are flushed, there may or may not be a little redness in the throat, and perhaps no other abnormal signs. After two or three days more the lymph nodes in the cervical triangles enlarge, followed by enlargement of the axillary and inguinal nodes. Occa-

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sionally there is nose-bleed but no hemorrhages in the mucous membranes or in the skin. The spleen may be palpable early in the course of the illness or it may not be detected before the end of a week or ten days. Until the blood has been examined one has in mind the possibilities of tuberculosis, of syphilis, of an acute Hodgkin's disease, or even of leukemia.

After ten days to three weeks the fever subsides, the symptoms improve rapidly, and in a few weeks the patient is symptomatically well although the enlargement of lymph nodes and spleen persists to some degree for a longer period and the blood returns to normal only after several months.

In none of the cases seen by us have we been able to trace contact from another case, and no secondary cases have been found in persons in intimate contact with our patients. Skin rashes were seen occasionally in our series, jaundice in one case, and as a rule prolonged weakness and lassitude have been prominent symptoms in the convalescent stage, sometimes for weeks after the acute stage of the disease.

Numerous articles in the literature touch on the hematological findings in the disease, but none of them have added much to the classical description of the blood by Downey in 1923, and later in 1936. His original colored plate with the description of the cells remains the best in any publication. Schwarz's12 and more recently Kracke's¹¹ description and colored plates are also very instructive and should be consulted for their fine details. These authors and others, including Tidy and Sprunt, call attention to the fact that in uncomplicated cases no noteworthy changes are to be found in the hemoglobin, red cells, or platelets. All of our cases have followed this rule. Most authors are agreed that in most cases a moderate leucocytosis is found, averaging about 12-15,000 in the active stage of the disease. Leucopenia is reported in occasional cases, especially early in the disease, by Longcope¹⁸, Kracke, Tidy, Farley⁸, and Davidsohn⁵, and very high counts only rarely. Counts over 30,000 are rare. The highest count recorded in American literature in an adult is 42,000 by McAlpin¹⁴. Tidy has never seen an adult case with oover 30,000. The highest count observed by him in a child is 35,000. Schwarz, however, quotes a case by Stepp and Wendt with 80,000 and one by Potter with 60,000. The highest leucocyte count in an individual case according to Schwarz occurs commonly on the fourth to twelfth day, but some may be at the maximum as early as the second or third day. Occasionally a leucopenia may persist for as much as three weeks (Steinfield and Goldberg)²¹.

The hematologic diagnosis is made chiefly by careful studies of blood smears. Very early in the disease a polymorphonuclear increase may be found, especially in epidemic cases (Sprunt²), and only by repeated counts will the characteristic lymphocytosis be observed. In nearly all cases, by the time a blood count is taken, a lymphocytosis will be seen, with a maximum on the fifth to the ninth day on the average. Most cases show a maximum from 60 to 80 per cent lymphocytes. Downey reports one case with 92 per cent and Ireland¹⁰ one with 97.5 per cent. turally, if counts are taken very early in the disease, lower lymphocyte counts will be found. Downey states that he had one case in which only normal lymphocytes were found, but he as well as most authors consider as the almost pathognomonic finding the presence of abnormal lymphocytes, usually in large numbers. These cells have been, and still are, poorly recognized by many persons examining blood smears, and because of this, erroneous diagnoses of leukemia have been too frequently made. important characteristic as pointed out by Downey is the fact that these cells are abnormal but not as a rule immature. usually constitute over half of the lymphocytes in the active stage of the disease, and in some cases nearly all of the cells may be of this type. Early in the disease they may be rare.

The cell seen as the characteristic type cell, called sometimes the "mononucleosis cell," is the Type I cell of Downey. These cells are pictured also in Schwarz's article and by Kracke. They are variable in size, often as large as a monocyte and sometimes as small as an intermediate sized lymphocyte, and commonly mixed sizes are seen in a single film of blood. The nuclei may be ovoid, indented, somewhat lobulated or irregular in shape, and are commonly situated eccentrically in the cell. Sometimes in the larger cells the nuclei may fill a large portion of the cell, but usually there is abundant cytoplasm. The nuclear pattern of

the majority of these cells is that of a mature cell with chromatin clumping and heavy strands and masses not sharply demarcated from the parachromatin. In some cells, rounded or angular chromatin blocking, particularly peripherally placed, causes great similarity to plasma cells. Schwarz, particularly, stresses the tending to plasma cell "umwandlung" in these cells. The fine sieve work chromatin pattern of the lymphoblast is not seen except in rare cells, and occasionally a case may show a small per cent of cells with considerable immaturity of the nucleus, corresponding to the Type III cells of Downey.

The cytoplasmic appearance is usually striking. One most commonly sees a wide rim of cytoplasm which stains deep blue by Wright's stain, much darker in shade than the color of a normal large lymphocyte. The spongioplasm is dense blue and in the background one sees a lighter staining, pale yellow hyaloplasm. The spongioplasm is more abundant at the periphery of the cells, so that the rim looks denser and the perinuclear zone paler than the mid zones. The distribution of spongioplasm and hyaloplasm is such that many of the cells appear vacuolated. This feature is particularly valuable as a diagnostic aid since it is so commonly found and so easily seen. Some cells also contain scattered fine or coarse red "azureophilic" granules, most commonly distributed in the inner layers of the cytoplasm. In some cells, Type II of Downey, the cytoplasm does not stain deeply and it is fairly uniform in staining.

In the active stages of the disease a moderate to marked shift to the left is seen in the neutrophiles, and in many cases relative mild monocytosis may be present. The eosinophiles do not disappear entirely and not uncommonly during convalescence are

slightly increased in number.

Several reports, especially those of Davidsohn⁵, Bernstein², and Stuart et al²³, verify the statement of Paul and Bunnell¹⁸ that the heterophile antibody reaction is of much confirmative value in the final diagnosis of infectious mononucleosis. As yet no one has offered a suitable explanation as to exactly why the serum of patients ill with this disease will usually agglutinate sheep red blood corpuscles in dilutions much higher than normal

blood. However, in the absence of injection of horse serum or serum sickness resulting from this, which conditions are also accompanied by slightly or moderately increased agglutinin titers, one can fairly safely agree with Bunnell³ that agglutination of sheep cells at a titer of 1:64 or above with Paul-Bunnell technique is indicative of infectious mononucleosis. Stuart et al state that agglutination at 1:80 is diagnostic and 1:20 suspicious. Certainly this laboratory evidence with the clinical and hematologic picture at hand should be sufficient to thoroughly clinch the diagnosis.

In interpreting the report of a heterophile test one must know the technique used, since if one varies the percentage of sheep cells and other factors, different titers will be obtained. Many of the articles in the literature report results of tests done by the original technique of Paul-Bunnell which was copied from Davidsohn's first method. Actually the titers reported by this method should be multiplied by 4 to correct for the true serum dilution. Davidsohn⁶ has modified his technique, Stuart has given a new method and Straus²² has outlined two techniques. All of these give much higher readings than the original method. However, titers obtained on normal bloods are proportionally increased also, so that in the long run one can safely use any technique one desires provided he knows the titer to be expected in normal bloods and bloods from patients with miscellaneous diseases exclusive of mononucleosis or serum disease. With the original Paul-Bunnell method of setting up and reading the tests which we have used in all our cases agglutinin content of less than 1:4 dilution is found in the vast majority of normal or miscellaneous bloods, and only very rarely does one find a titer as high as 1:16. Bunnell reports a case of arthritis with a titer of 1:32 and quotes a case of Davidsohn with a titer of 1:64 in a case under treatment with insulin.

Furthermore the warning of Bernstein concerning interpretation of border line titers appears to be well founded. It is not uncommon for bacterial agglutinins to be increased non-specifically by febrile response to some unrelated infection. Consequently it may be possible that patients with a relatively high

normal sheep-agglutinin content of the blood may have this titer increased into the zone where one might consider it suspicious or of diagnostic import. For example one might normally have a titer of 1:8. This might be raised to 1:32 by some disease having nothing to do with mononucleosis, and some question might be raised as to the future course of the clinical picture. On the other hand a person who gives a normal antibody content of 1:1 might show only 1:16 or 1:32 in the early stages of an attack of acute mononucleosis even though his titer is multiplied by 16 or 32. In order to definitely prove that the agglutinins present in the serum are due to infectious mononucleosis, Bailey and Raffel¹ recommend testing before and after absorption by raw or boiled ox cells. Davidsohn recommends both ox cells and guinea pig kidney. If the antibodies are due to infectious mononucleosis, they will be absorbed by the ox cells whereas the increased so-called "normal" agglutinin is not absorbed. The agglutinin found in serum sickness is also absorbed by ox cells as well as by guinea pig kidney, but those of infectious mononucleosis are not absorbed by the latter. These additional tests are hardly necessary in the average case, which shows the usual clinical and hematological picture, but may be helpful occasionally when one is forced to do his best to make an immediate answer as to what certain titers mean in the test.

In early cases where the clinical and hematologic pictures are not completely developed, agglutinin titers of 1:16 and 1:32 must be considered of value, and above all, further blood counts and agglutination tests run, just as one does in a suspected case of typhoid fever. As a rule the statement of Bunnell is quite true that the development of the heterophile agglutination reaction runs parallel with the pathological lymphocytosis, and that as the pathological lymphocytes decrease the agglutination titer decreases. However, an occasional case is seen where this parallelism does not hold. Bernstein, for instance, found an agglutination of 1–256 on the seventh day of illness in a case where on the day before no pathological lymphocytes were found, 45 per cent of the cells, however, being normal large lymphocytes. Stuart et al also cites a case where the heterophile reaction was

negative on the third day of the illness although the blood picture was that of infectious mononucleosis. Later the heterophile reaction became positive at 1–2560 dilution. Also there are a small per cent of cases hematologically and clinically infectious mononucleosis which consistently gives a negative heterophile test. Davidsohn therefore believes there are two varieties of the disease—the seropositive, and the seronegative.

The agglutination reaction is stated by Bernstein to become positive about the sixth day of the illness. McAlpin, however, found a positive test of 1:128 dilution on the third day of the illness of one patient, and Bunnell reports two cases with 1:64 and 1:128 titer on the fourth day, and one with 1:256 on the fifth day. Our own experience has been to the effect that positive tests have been found as early as the fifth day. Some discrepancies may be explained on the difficulty of determining the exact day of onset of the disease. Several reports in the literature attest to the fact that the heterophile reaction is a fleeting one and that the agglutinins do not persist like the agglutinins following typhoid fever, for example, long after the disease had run its course. The heterophile antibodies tend to disappear as the patient gets better and as the blood comes back to normal, and seldom persists after a period of a few months unless the disease runs a protracted course or the patient suffers a relapse, which occurs not infrequently. In a rather careful study on a series of 10 cases, Davidsohn⁵ found that the heterophile titer reached normal after 56 to 296 days—an average of 119 days. serologic recovery was slightly slower than the hematologic recovery and this in turn was slower than the clinical recovery.

In the last three years, 42 cases have been studied by the authors. In none of these was there any noteworthy changes in the hemoglobin content or the red cell counts in the blood. The white cell counts ranged from 3,700 to 22,000 with an average of 13,000 to 14,000 (table 1). As a rule, the low white counts were found in the early stages of the disease and later normal or higher total white counts were seen. In 5 cases, counts below 5,800 were found. Persistent leucopenia was not seen, nor have we ever seen this occur. On a whole the white count approached

normal values as the patient clinically recovered from the symptoms of the disease. The hematologic return to nearly normal figures was quite rapid in some cases, considerably faster than in some of Davidsohn's cases.

Study of blood smears showed relative and absolute lymphocytosis in all cases. A polymorphonuclear increase was not present, even in cases seen very early. Occasional case showed as low as 32, 34, 44 and 45 per cent lymphocytes when first seen, but without exceptions these percentages were increased in later counts. On the average, maximum per cents of 65 to 70 per cent lymphocytes were found. The highest count was 85 per cent. In all cases but one, case 6, including those with the lowest lymphocyte counts, some of the typical abnormal lymphocytes were found on the first examination, and in all cases they were found on repeated examinations. On the average about one-third to one-half of the total lymphocytes at the height of the disease were abnormal types as described above, and occasionally as many as 80 per cent. The cells designated as abnormal lymphocytes corresponded to the classical descriptions of Downey, Schwarz, and others. Downey's type I cell with abundant deep blue, often vacuolated cytoplasm was seen in all cases. No attempt was made to separate the three types of Downey cells. In most of the cases some cells with fairly young nuclei were seen, and occasionally as high as 2 per cent stem cells were found.

A normal or slightly increased monocyte count was usually observed, in only two cases exceeding 12 per cent. The neutrophiles were reduced as the lymphocytes increased and characteristically showed a definite shift to the left. There were nearly as many non-segmented neutrophiles as segmented types, and occasionally more. Toxic degeneration in the cytoplasm was seen in varying degrees, usually not as a prominent feature in the slides.

We have failed to find vacuoles in nuclei of some of the lymphocytes as described by Osgood¹⁷. All our smears have been made from non-oxalated blood and we cannot say at this time whether the vacuoles in question are due to the factor of oxalation or not. We have corroborated by supravitally stained prepara-

TABLE 1
INFECTIOUS MONONUCLEOSIS

											HAW				SHEEP	shbep cell addlutining serum dilutions	LUTININ	SERUM	DILUTIO	NB		
GAAD	XXS	MON	OCCUPATION	DATE	AFTER ONSET	W.B.C.	POLYS POLYS NOW SEG.	POLTS	EOSINOPH BASOPHIL	MONOCYT	TOTAL LT	VRACHE	154	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:512 1:1024 1:2048	1:2048
R			Student	10/21/36		17,500	36	64	0	000	52	38	++	#	+	1	1	1	1	1	1	1
2 H. O.	M	18		3/30/32	5 days	13,400		22	0	-	32	15	+++	‡	+	+1	1	1	1	1	1	1
				4/ 1/35		10,200		12	0	00	_	16										
1				5/11/35		11,600	2	9	0			0										
P. E.	M	18	Actor	10/ 9/35	5 days	21,300	-de	124	0		72	24	+++	++++	+++	‡	+	ı	1	1	ı	1
				10/10/35		13,600		101	9	-	-	42										
				10/11/35		14,100	= 8	0 5	0 0	90 0	71	900										
	-	-		10/17/30		11,200	- 1	2		_	_	207	:									
5.8	14	18	None	9/ 2/36	5 days	15,900	25		0	_	_	41	None	None made								
				9/ 6/36		8,800	38	_	0			+++										
ß.	_	19		10/ 7/36	9	7,200	21	21 (0	_	_	38	++++	++++	++++	++++	++++	++++	++++	+++	+	+
6 P. L.	M	24	Electrician	7/30/35	6 days	4,700	70		53	_		0										
				8/2/35		6,400	13	13	0	_	_	Few	++++	+	+1	1	ı	1	1	1	1	1
				8/ 6/35		9,000	14	00	0	=======================================		++++	++++	++++	+	+	+	#	ı	1	1	1
				8/ 9/35		18,700	16			***	_	++++	++++	++++	+	+	+	+	#	1	1	1
				8/17/35		7,400	_			-			+	‡	‡	+	+1	1	ı	1	ı	1
				6/ 9/36		7,300	55		1 0	=	33	0	+	1	1	1	1	1	1	1	1	1
H	54	48	Housewife	5/10/37	6 days	7,200	_	20	0	2	46	22	++++	+++	++++	++++	++++	++++	+	‡	+	ı
M.C.		21		6/24/35	1 week	4,100	_	22	3	64	45	18	+	+	+	+	+	+	1	1	1	1
				6/27/35		006'6	16	10	3	-	9	36										
H.	F4	21	Student	10/15/34	1 week	15,900	18	10	0 1	-	89	53	+++	+++	+	+	+	+1	1	1	1	1
10 S. S.	Ē4	-	Housewife	7/19/35	1	5,500	-	_	2 0	10	_	20										
L' L	M	_	Student	11/29/36	1	20,000	20	_	-	_		+++	++++	+++	++++	++++	+++	+	+	+	+	#
0.0	54	24	Stenog.	11/21/35	1	7,800	164	18	0			23	++++	+++	++++	++++	++++	++++	+	+	+	ı
M.	M		Banker	2/22/38	6	9,500	32	-	1		43	++	++++	+++	++++	++++	+	++	+	+	1	1
				2/24/38		17,300	24	_	0 0	_		+++										
	_	-		3/ 6/38		12,500	20	_	_	-	_	+++										

1		1		ı		1			1		1	1							1	ı	1		1	1		1	1	1	ı	i	1	ı	1		1	ı	1
1		1		1		1			1		1	1				105	99	30	1	1	1		1	ı		+	ı	1	1	1	1	1	1		1	#	1
1		ı		H					1		+	+							1	1	1		1	+		+	1	1	1	1	1	1	1		1 -	+	+
1		1		+		+			+		+	++							1	ı	1		1	+		+++	1	1	1	1	1	ı	1		1 ;	+	+
1		1		+		+			+		+	+++				•1	•1	*1	1	1	1		+	+++		++++	+	1	1	ï	1	1	1		1	++++	++++
1		1		+		+			+++		+++	++++				ı	1	1	1	+	1		+	+++		++++	+++	1	1	1	1	1	1		+	++++	++++
1		+		+++		+++			++++		++++	+++				1	+	+	+	‡	1		+	+++		+++	+++	ı	1	1	1	1	1		‡	+++	++++
1		+	-	++++ ++++ ++++ +++		++++ +++++++	_		+++		+++	++++				1	+	+	+	+	i		+++	+++		+++	+++ +	+	+	+	++	1	ı		+++	+++	+++
1		+++	-	++++		++++			++++		++++	++++		_		1	+	++	‡	+++	1		+++	++++		++++	+++	+	+	+++	+++	+	1			+++	+++
+		++++++	-	++++		++++			++++		+++++++	++++				+	+++	+++	+++	++	+		++++	++++		++++	++++	+++	+++	++	+++	+	1		++++++	++++	++++
24	41	71 +		+	10	32 +	31	20	36 +			+ 23	25		++	+++	+	+	+	_	0	63	37 +	+ 09	9	+	+	T	1	_	1		32	++	++	+ 07	+ 01
7	_	_	Т_	-	36	72	99	67	62		22	24	26			8				78	16	89	63	11	82			_	_	_	_		99	#	26	89	11
10	10	10	10	-	10	03	*	2	*		90	11	12		91	00				64	10	60	9	+	63								12	1	12	10	-
0	C4	0	0	,	0	0	0	0	0		-	-	-		-	0				0	0	0	0	0	0								0	-	-	0	0
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45	34	5	- 53		37	14	17	7	16		18	24	24		60	31	-						12											33			
5,800	8,100	2,800	12,600		7,700	16,600	22,000	9,100	8,900		7,500	9,000	6,800	3,700	5,000	12,000				17,000	8,200	12,800	5,800	13,000	12,000								11,400	9,800	6,300	12,000	12,000
10 days				10 days		10 days			10 days		10 days	11 days				13 days				2 weeks			2 weeks	2 weeks									2 weeks		17 days	19 days	3 weeks
5/22/36	6/2/36	6/12/36	6/20/35	6/27/35	11/3/35	11/8/35	11/17/35	11/21/35	6/8/37	6/11/37	2/14/38	11/24/37	11/27/37	4/ 6/36	4/ 9/36	4/11/36	4/18/36	4/24/36	5/15/36	3/23/35	6/8/35	6/19/36	6/23/36	12/30/36	1/8/37	1/14/37	1/28/37	2/12/37	2/25/37	3/13/37	3/27/37	4/27/37	12/11/36	8/20/35	8/22/35	10/19/37	1/28/36
Actor			School boy		Student				Student		Lawyer			Student						Nurse		Housewife		Student									School boy	Salesman		ach	Student
30			12		18				g		33	28		8						21		21		22									10	43		38	16
M			M		×				P4		M	í4		1						[±		í-		-									M			M	M
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14 H.			15 L. W		16 G. W				17 O.B.		8 J. R.	19 J. S.	_	20 R.		_			_	21 E. F		22 I. C.	_	23 L. M. M	_	-		_	_		_	_	24 B. B	5 L.O.	B.	26 V. C.	7 B. 1

TABLE 1—Concluded

	1:2048				1	1	1	1	H		1	i			ì			1	1	
	1:512 1:1024 1:2048				1	+	1	1	+		1	1			1			1	+	
90	1:512				+	+	1	+	+		ı	+			ı			1	+++	
ILUTION	1:256				+++	++	1	+	++		1	++			1			1	+++	
SERUM D	1:128				++++	++++	1	+	++		1	++			1			1	++++	
sheep cell agglutinins serum dilutions	1:64				++++	++++	+	++	++++		ı	++++			+1			1	++++	
ELL AGGL	1:32				++++	++++	+	++++	++++		+	++++			+			1	++++	
SHEEP CI	1:16				++++	++++	+	++++	++++		+	++++			+			1	++++	
	1:8				++++	++++	+	++++	++++		++++	++++			++			1	++++	
	134				++++	++++	+++	++++	++++		++++	++++			++++			+	++++	
	гамьна Увиони	324	+++	+++	634	20	21	40	90	Rare		21	Rare	Rare	+++	19	6	15	26	1
BHTM	TOTAL LY	75	22	554	85	20	49	26	83	61		62	27	49	11	37	40	55	61	27
83	MONOCAL	60	10	34	2	10	6	12	-in	-		6	NO	-	-	60	2	9	60	2
82	пичовая	0	0	0	-04	23	0	-	0	0		0	-	0	63	0	0	-	0	0
STI	EOSINOPE	0	-	_	-44	0	0	-	1	63		7	10	2	0	0	1	9	0	2
,	HOLTS NON SEC.	-		_	+	10	12	13	149	-		13	03	~	_	43	25	6	11	2
		22	3	4			_	_		30	provident		62	80	8	2	22	63	10	57
ED	POLTS				NO	18	30	17	-			14				17	CA	53	64	
a a	W.B.C.	16,700	8,800	5,900	24,600 5	8,100 18	006	12,000 17	8,200 1	17,100			7,650	6,300	20,000		7,100 2			8.500
C S	W.B.C.	3 weeks 16,700			009	100	-	12,000	00	17,100	6 weeks	weeks 9,250	7,650	6,300	7 20,000					
da	TNEMDES	weeks 16,	80		3 weeks 24,600	3 weeks 8,100	26 days 11,900	Several weeks 12,000	Several weeks 8,	2/23/37 17,100	9	7 weeks 9,250	2/20/36 7.650		7 20,	006'9	7,100	10,400	7 16,200	00
C S	AFTER ONSET AFTER ONSET W.B.C.	3 weeks 16,	80	5,	3 weeks 24,600	3 weeks 8,100	26 days 11,900	Several weeks 12,000	Several weeks 8,		9	7 weeks 9,250			7 20,	006'9	7,100	11 weeks 10,400	7 16,200	3/38
QZ.	DATE TIME OF TEST AFTER ONSET OF HERE	11/16/35 3 weeks 16,	80	5,	Post Office 4/19/38 3 weeks 24,600	5/ 1/37 3 weeks 8,100	Nurse 5/23/36 26 days 11,900	Several weeks 12,000	Engineer 2/ 9/37 Several weeks 8,	2/23/37	9	2/ 4/36 7 weeks 9,250			6/16/35 7 20,	Grocer 2/ 1/35 6,900	7,100	11 weeks 10,400	5/11/38 7 16,200	3/38
dz.	OCCUPATION DATE TIME OF TEST C. AFTER ONSET C. THE TEST CONSET C. THE TEST CONSET CONS	Housewife 11/16/35 3 weeks 16,	80	5,	M 21 Post Office 4/19/38 3 weeks 24,600	M 25 Office 5/ 1/37 3 weeks 8,100	F 34 Nurse 5/23/36 26 days 11,900	M 5/ 7/37 Several weeks 12,000	M 23 Engineer 2/9/37 Several weeks 8,	F 9 School girl 2/23/37	9	School girl 2/ 4/36 7 weeks 9,250			26 Chemist 6/16/35 7 20,	37 Grocer 2/1/35 6,900	7,100	11 weeks 10,400	Saleslady 5/11/38 7 16,200	3/38
dz	OCCUPATION DATE TIME OF TEST AFTER ONSET C. THE AFTER C. THE AF	50 Housewife 11/16/35 3 weeks 16,	80	5,	M 21 Post Office 4/19/38 3 weeks 24,600	M 25 Office 5/ 1/37 3 weeks 8,100	F 34 Nurse 5/23/36 26 days 11,900	M 5/ 7/37 Several weeks 12,000	23 Engineer 2/ 9/37 Several weeks 8,	F 9 School girl 2/23/37	9	13 School girl 2/ 4/36 7 weeks 9,250			Chemist 6/16/35 7 20,	H. M 37 Grocer 2/1/35 6,900	7,100	11 weeks 10,400	25 Saleslady 5/11/38 7 16,200	3/38

· Icteric index.

tion in a few cases the report by Gall⁹ that only a small portion of the lymphocytes in the bloods from mononucleosis cases show refractive granules. Gall found that refractive granules, present in about one-third of the lymphocytes in other lymphatic diseases and in the normal state, were not present in higher than 15 per cent of the lymphocytes in infectious mononucleosis. This procedure is tedious compared with other laboratory procedures used and we doubt that it will gain popular favor except in those laboratories where considerable numbers of wet film mounts are commonly studied.

Study of table 1 shows that a definitely positive heterophile test of 1:32 dilution or higher was found in all cases except four 5 days or more after the onset. Case 1 showed on the third day after onset a reading of 1:16. The most of the cases studied between 1 and 2 weeks of onset showed titers averaging about 1:256 to 1:512. The highest reaction (1:2048) was in case 5, a young man who suffered from acute abdominal pain and fever to such a degree that his surgeon removed his appendix. Markedly enlarged lymph nodes were found in the mesentery. One of these was removed and showed lymphocytic and reticular cell proliferation typical of mononucleosis (Downey). Case 24, a boy of 10, showed a typical clinical picture of mononucleosis of two weeks duration, with generalized lymphadenopathy, subsiding sore throat, and a typical blood picture with 32 per cent abnormal lymphocytes, but no heterophile antibodies were demonstrated. Case 14 was characterized clinically by fever lasting over a month but with no node enlargement except moderate cervical involvement after 3 weeks after the onset. Increased heterophile agglutinins were not demonstrated at 10 days after the onset, but 3 weeks later a slight positive test of 1:32 was obtained. Case 13, likewise free from node involvement but showing fever for 3 weeks, gave a 1:512 reading 9 days after the onset. Case 20 showed a marked jaundice during the course of the illness. This case showed a typical blood picture, but at the end of 13 days the heterophile reaction was 1:4 only. A week later it had increased to 1:32 but did not go higher. No absorption tests were done with ox cells or guinea pig kidney in any of the above cases.

TABLE 2
INFECTIOUS MONONDUEDSIS

	HIGHEST		TIME OF TEST			9E	EEP CELL AC	BOLUTINATI	ON TEST AN	BHERP CELL AGGLUTINATION TRST AND OX CELL HEMOLYTIC TEST	HEMOLYTIC	TEST		
CASE	WBC	COUNT	AFTER		1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
А. Н.	20,000	18	weeks 7½	Sh.	++++++	++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	++++++	+++++	++++	1+++	1 ‡	1 +
M. A.	7,200	84	7	Sh.	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	+++++++	++++	#+	ı +	1+	1 1	1.1	1 1
R. C.	15,500	64	60	Sh.	++ ++ ++ ++	+++++++++++++++++++++++++++++++++++++++	+++++++	+++++	# +	1 +	1+	1 1	1.1	1 1
D. N.	17,700	85	9	Sh. Ox	#+++	+++++++++++++++++++++++++++++++++++++++	++++	1 + +	1 +	1 +	1+	1 1	1 1	1 1
E. C.	Febrile	Febrile illness, mononucleosis	not	Sh. Ox	#+	1.1	1 1	1 1	11	1 1	1 1	1 1	1 1	1.1
M. C.				Sh. Ox	1+	11	1.1	1 1	1 1	1 1	1.1	1.1	1 1	1.1
R. R.				Sh.	++	++	1.1	1 1	1 1	1 1	1 1	1 1	1 1	1.1

Sh. = sheep cell agglutination test. Ox = ox cell hemolytic test. Table 2 demonstrates a short series of 4 cases of infectious mononucleosis and 3 controls in which simultaneous sheep cell agglutination and ox cell hemolysin tests were done. The results showed that the ox cell hemolysin tests were positive in greater dilutions than the agglutination test.

Considerable difficulty of interpretation was presented by a series of febrile cases (table 3), which presented blood pictures very much like or indistinguishable from infectious mononucleosis but which clinically were something else. Case 1 was a case of acute ethmoid sinusitis with no lymph node enlargement or sore throat. There were 19 per cent abnormal lymphocytes and the heterophile test was negative. Case 2 was a chronic alcoholic who entered in coma resulting from alcohol and overdosage of barbiturates. Nine per cent abnormal lymphocytes out of a total of 55 were present. No sore throat or lymph node en-The heterophile test was entirely negalargement was found. tive. Recovery was prompt. Case 3 was a case of thrombocytopenic purpura (reported elsewhere with similar cases by Minot¹⁶) who developed fever of 101° to 103° without sore throat, enlarged lymph nodes or other apparent source of infection. High abnormal lymphocytosis, at one time 48 per cent, persisted for over a month. Heterophile reactions were negative. A few abnormal lymphocytes were found in the blood of case 4, a boy with tracheobronchitis for 4 weeks, fever 99.5° to 100°F., but no evidence of lymphadenopathy and a negative heterophile test. Case 6 was a brewer who had been feeling under par for several Following asphyxiation from fumes from some unknown cleansing fluid in a closed beer vat, he developed cyanosis, profound prolonged dyspnea (apparently from methemoglobinemia), a fever of 100° to 102°, but never any lymph node enlargement. At one time as high as 55 per cent abnormal lymphocytes were found, and two heterophile tests were negative. We do not know how to classify this case. Case 7 was a child who suffered lower abdominal pain of dull type for two weeks, showed only a slight fever, never any enlarged nodes or spleen and showed 12 abnormal lymphocytes and a questionable heterophile test. In this case we probably could have proved whether

TABLE 3
LYMPHATIC REACTIONS

	1:64 1:128 1:256 1:512	1	1						1	1			1	1		1		1	1	
OTTO)	1:25	1	1						1	1			1	1		1		1	1	
M DIE	1:128	1	1						1	1			1	1		1		1	1	
SERU	1:64	1	-1						I	1			1	1		1		1	1	
NINB	1:32	1	1						1	1			1	1		1		1	+	
BGLUT	1:16	1	1						1	1			+	1		1		1	++	
SHEEP CELL AGGLUTININS SERUM DILUTION	1:8	+	1						+1	1			++	1		+		+	+++	
BHE	1:4	++	1						+	+			+++	1		+		++	++++	
7	ABNORMA LTMPHS	19	6			M	•		48	19	Rare		Rare	12		27	55		12	AC.
SHAP	TOTAL LT	57	55			10	2		28	61	25		44	69		99	89		36	40
93	MONOCAL	10	13			1	•		က	00	16		=	00		_	-		5	9
83	вуворны	0	0			0			-	0	0		0	0		0	0		0	-
ILES	HORINOBH	-	10			1			6	-	4		-	0		01	-		-	0
POLTS	NON BEG.	13	00			2	3		25	15	20		4	13		22	13		25	7
da.	POLTS	18	19			96			4	15	63		4	10		6	17		33	46
	W.B.C.	6,100	9,600			200	6,000			9,500	5,900		5,800	4,100		20,300	17.700		8,900	7 200
TIME OF	TEST AFTER ONSET	Ethmoid	sinusitis Alcohol-	ism bar-	bitura-	Purming	hemor-	rhagica			Hyper-	thyroid-		4 weeks		Several	Weeks		2 weeks	
	DATE	10/ 6/34	1/ 5/35			4/16/25	7/ 10/ 90		4/20/35	5/16/35	12/ 4/36		12/8/36	1/29/37		5/21/36	5/27/36	6/ 1/36	12/31/37	1/3/38
	OCCUPATION	6-	Retired			Margaria	IN OTRICE				Housewife 12/ 4/36			School	boy	Brewer			Student	
	ADA	23	M 39			- 9					21			M 12		M 37			13	
	XIS	1				F	4				H								H	_
	CASE	N. T.	M. B.			ρ	-				B.			A. B.		W. I.			H.B.	
		z	M			р	i				4			A.		3			H	

it was true infectious mononucleosis if we had used the absorption tests recommended by Davidsohn and Stuart.

These cases demonstrate that abnormal lymphocytes may occur in other conditions than infectious mononucleosis, although as a rule not many are found. Certainly here, as in the whole field of medicine, the laboratory evidence should be interpreted in the light of the clinical picture presented by the patient.

SUMMARY AND CONCLUSIONS

A review of the hematologic features of the blood in cases of infectious mononucleosis is given with particular emphasis on the characteristics of the abnormal lymphocyte, the so-called "mononucleosis cell." The heterophile antibody reaction is discussed and its field of use in diagnosis stressed.

A summary of 42 cases observed by the authors is presented. Leucopenia was found in 5 cases when first seen. Abnormal lymphocytes were seen in all cases, no matter how early observed. The heterophile reaction was negative in 4 cases, and positive strongly in the majority.

A series of patients with lymphatic reactions simulating mononucleosis in their blood pictures is presented. The usefulness of the heterophile test is shown in these cases, but the fact is brought out that absorption tests with beef cells and guinea pig kidney are necessary to evaluate the findings of this test in some cases.

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XANTHOPROTEIC AND INDICAN STUDIES ON THE BLOOD IN RENAL INSUFFICIENCY*

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The purpose of this paper was to investigate the diagnostic and prognostic value of the xanthroproteic reaction and blood indican determination in cases of renal insufficiency.

THE XANTHOPROTEIC REACTION IN RENAL INSUFFICIENCY

In 1924 Becher¹ described the yellow color obtained when nitric acid reacts with phenol derivatives in solution as the xanthoproteic reaction. He noted that a positive reaction results when aromatic amino-acids (tyrosine, tryptophane, and phenyl oxyproprionic acid) and phenols and cresols are present in the circulating blood and body fluids². He also observed that the amino-acid group is insoluble in ether, and that the phenol group is ether soluble. On this difference, the presence of each group separately in the blood could be determined by hydrolysis of the serum and extraction with ether. Normal value for the xanthoproteic reaction done on whole serum without preliminary hydrolysis and ether extraction was given by Becher as 25. This figure represented a measure of the percentage of color found in a positive test by comparison with an 0.3874 per cent potassium bichromate solution.

Impressed by the clinical resemblance of chronic phenol poisoning to uremia, Becher³ wondered whether many of the symptoms of uremia might be due to retention of intestinal putrefactive products including phenol and indol. This speculation was soon confirmed by finding in uremia positive values for the xantho-proteic reaction which ranged from 83 to 250. Further, in uremia

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the ether soluble group was the one greatly increased in contrast to a rise of the ether insoluble group in other diseases such as atrophies and cirrhoses of the liver. Curiously, in uremia of acute nephritis, the xanthoproteic reaction did not yield increased values.

Since its introduction by Becher, the xanthoproteic reaction has been used singly and in combination with blood indican determinations as a method of clinical study in the diagnosis and prognosis of renal insufficiency by workers on both continents.

In Europe, Klein¹⁰ published the first clinical confirmation of Becher's data in renal insufficiency. Altering the colorimetric standard to an 0.01 per cent solution of tyrosine, Kaemmer⁸ also obtained corroborative results and recorded high readings in liver damage, pneumonia, and thyrotoxicosis with high basal metabolic rates. Irdelp⁷ placed the normal value between 15 and 35, and demonstrated positive reactions with increased values in all types of renal insufficiency. In Rasmussen's¹⁶ series however, the xanthoproteic reaction was not elevated in cases of uremia with an acute onset; the reaction was proven positive following administration of large doses of salicylates to patients. Mueller¹⁴ discovered that up to a few hours before death the xanthoproteic reaction remained normal, and shortly before and after death, there occurs an increase in the aromatic substances in the blood.

Up to the present Steen¹⁷ has presented the only published American study on the xanthoproteic reaction. He analyzed 210 tests carried out on blood and body fluids from living patients and from autopsies. An arbitrary set of standard dilutions of potassium bichromate solution was substituted for the colorimetric method of grading results. All patients who showed high values for the xanthoproteic test died of renal insufficiency. Strongly positive results were recorded in both blood and body fluids. Recently Mason¹² produced uremia in dogs, and found an increase in the phenol content of blood and spinal fluid.

BLOOD INDICAN DETERMINATION IN RENAL INSUFFICIENCY

In 1915 Jolles made a new modification of the Obermayer-Popper reaction for the quantitative determination of indican in

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urine. Two years later Haas⁵ modified the method of Jolles and applied the consequent procedure to the determination of indican in blood serum. He appraised the normal value for blood serum indican to be 0.026 to 0.08 mgm. per cent. In cases of kidney disease especially in uremia, Haas found values 30 to 60 times more than normal. Certain of the authors previously mentioned, in their work with the xanthoproteic reaction in uremia, concluded that the reaction tended to run parallel with the blood urea nitrogen and blood indican concentrations.

Monias and Shapiro¹³ determined the blood indican in 104 patients and estimated 0.015 mgm. per cent as the upper limit of normal. In 31 cases the indican value ranged from 2.5 to 5.0 mgm. per cent. All of these died of uremia; in five however, only slight rises of the blood urea nitrogen were noted. Cases with low indican values recovered or died from extra renal causes. Monias and Shapiro also devised an artificial standard and a formula for calculation of the indican value.

Livierato and Simoneto¹¹ gave 0.16 to 0.64 mgm. per cent as the normal value for indican in the blood. They found hyperindicanemia in such conditions as: renal insufficiency, intestinal obstruction, diseases of the liver, and other conditions associated with considerable destruction of albumin as in empyema, and lung abscesses. In renal insufficiency the increase was more marked than in any other disease.

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Keith and Wakefield⁹ determined the blood indican in cases of nephritis, hypertension with renal insufficiency, pyelonephritis, and polycystic kidney, and deduced from their study that the test possessed more prognostic than diagnostic value.

Polayes and Eckert¹⁵ examined the blood indican in patients with and without renal disease. Indicanemia was always accompanied by an increase in the non-protein nitrogen of the blood. A rising indicanemia coupled with a fall in the urinary output of indican, was significant of a rapidly fatal prognosis.

Wells¹⁸ states that the blood indican in normal subjects reaches 0.05 mgm. per cent and may rise to a level of 0.2 mgm. per cent in cases of uremia.

The foregoing review of the available literature on blood indican determination, discloses disparities in the normal and pathological

values for blood indican as furnished by various authors. We believe that such disparities may be due in part to the inherent limitations of the blood indican determination itself, and to the variation of different procedures used in obtaining quantitative results. Monias and Shapiro, and Polayes and Eckert followed Jolles' method which requires 5.0 cc. of blood serum, and employed the artificial standard of Monias and Shapiro. On the other hand, Haas, Keith and Wakefield, and Livierato and Simoneto worked with a modification of the Jolles method in which 1.5 to 2.0 cc. of serum are utilized. Further, in the performance of any type of blood indican determination, the amount of filtrate obtained from the original sample of serum is not always constant. The quantity of filtrate from a given amount of serum varies inversely with the amount of protein precipitated from the serum. The filter paper itself withholds varying amounts of fluid for which no correction can be made. For these reasons we feel that a perceptible percentage of error in the quantitative determination of blood indican must be taken into account. In an attempt to reduce the degree of error, we have introduced in our study a new modification of the Haas-Jolles procedure which uses a definite aliquot of the material and thus insures examination of a constant amount of serum in every determination (see below under Methods).

METHODS

In our study the xanthoproteic reaction and blood indican determinations (Haas-Jolles and author's modification) were carried out simultaneously.

Procedure for xanthoproteic reaction (Becher). Five cubic centimeters of blood serum are placed in a test tube. Then 5.0 cc. of 20 per cent trichloracetic acid are added. The resulting solution is shaken well and filtered. To 2.0 cc. of the filtrate are added 0.5 cc. of concentrated nitric acid (sp. gr. 1.4). The acidified filtrate is now boiled for 30 seconds over a flame and immediately cooled under running water. One and one-half cubic centimeters of 33 per cent sodium hydroxide are added and a yellow color of varying intensity appears. The color reaction is quantitated by comparison with the color intensity of an 0.3874 per cent solution of potassium bichromate diluted 1:10. The results are ex-

pressed in percentage of color intensity of the unknown as compared with the diluted standard:

 $\frac{\text{reading of standard}}{\text{reading of unknown}} \times 100 = \text{Xanthoproteic reading}.$

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Procedure for blood indican determination (author's modification of the Haas-Jolles method with Monias and Shapiro artificial standard and formula). Two cubic centimeters of serum are placed in a test tube. To this amount, 2.0 cc. of distilled water and 4.0 cc. of trichloracetic acid are added. Here, in the precipitation of the serum protein, the serum is diluted 1:4. After filtration, 4.0 cc. of the filtrate are pipetted off and the test is performed on this definite amount with the knowledge that it contains 1.0 cc. of original serum. To 4.0 cc. of the filtrate, 7 drops of 5 per cent alcoholic solution of thymol are added and the mixture shaken. Then follows the addition of an equal volume of Obermayer's reagent (1000 cc. of hydrochloric acid, Sp. Gr. 1.19, and 2.0 grams of ferric chloride). The tube is allowed to stand for 2 hours at room temperature, or it may be heated in a water bath at 40°C. for 1 hour. Two cubic centimeters of chloroform are then added and the contents of the tube thoroughly mixed. The tube is now allowed to stand until the chloroform settles out in the bottom of the tube. To quantitate the results, the artificial standard of Monias and Shapiro corresponding to 5.0 mgm. per cent of indican is employed (6 capillary drops of 1 per cent aqueous solution of gentian violet; 3 drops of 1 per cent aqueous solution of eosin; 10.0 cc. of 94 per cent alcohol; and 30 cc. of distilled water). The formula for calculation as given by Monias and Shapiro is:

 $\frac{\text{reading of standard} \times 0.5}{\text{reading of unknown}} \times 10 = \text{mgm. indican/100 cc. blood}$

Monias and Shapiro dissolve the indican in amounts of chloroform equivalent to the amount of serum used at the beginning of their test. In view of the fact that in our method, we take a constant serum content of 1 cc. and as a solvent 2 cc. of chloroform, the concentrate becomes just one half of that present in the serum. For this reason, the Monias and Shapiro formula must be modified for our method, so that the multiplying factor is 20 instead of 10.

COMMENT

On the basis of a survey of the foregoing tables in general, and of a study of individual cases, we are able to make several deductions in regard to the diagnostic and prognostic value of the xanthoproteic reaction.

In the first place, a high xanthoproteic reaction (above 70-80

TABLE 1
Low Values for Xanthoproteic Test

CASE	CLINICAL DIAGNOSIS	UREA	CREAT	XANTEO	INDICAN	REMARKS	RESULTS
1	Undetermined	26		33	Neg.		Disch. imp.
2	Hypert. prostate, pyelonephritis	88	4.5		Tr.	Operation and im- prove- ment	Disch. imp.
3	Extrav. of urine	40	1.3	62	Neg.	Cystotomy	Death-not due to renal insuff.
4	Pyelonephritis	81		28	Neg.		Disch. imp.
5	Hypert. prostate, pyelonephritis 12/5			111	2.9	Test done after 36 h.	
	12/18	22		35	Neg.	Imp.	Disch. imp.
6	Diabetes Mell.	34		35	Neg.		Death—perfor. ulcer
7	Atr. arthritis	15		44	Neg.	77	D' 1 '
0	Peptic ulcer	43		44	Tr.	Hemor- rhage, de- hydration	Disch. imp.
9	Diabetes Mell.	16			Neg.		
10	Acute nephritis	15		38			Disch. imp.
11	Acute nephritis	20		32	Neg.		Disch. imp.
12	Malig. neutro- penia	28		34	Neg.	Dehydrated	Autopsy—Stomati- tis, general anemia
13	Ac. rheumatic f.	13			Neg.		
14	Dehydration	50		36	Tr.	Dehydra- tion	Disch. rec.
15	Ch. glom. neph.	34		29	Neg.		Disch. imp.
16	Ch. glom. neph.	52		30	Tr.	Dehydra- tion	Disch. imp.
17	Pern. anemia, acute nephritis, liver abscess	173		50	Tr.	Drowsy, restless	Autopsy—Common duct stone, liver abscess, subacute glom-nephritis
18	Subacute nephritis, nephroses	42		25	Neg.	Nephrotic ed. low proteins	Died—pneumonia, no autopsy
19	Malig. hyperten.	136		71	1.2	Comatose	Died-no autopsy
20	Acute glom. ne- phritis, lobar pneumonia	12	1.2	15	Neg.	Convulsions	
21	Tabes dorsalis, cord bladder, pyelonephritis	81	6.4	55		No symp- toms	Autopsy, pyelone- phrosis, lobar pneumonia

 ${\bf TABLE~2}$ High Values for Xanthoproteic Test

CASE	CLINICAL DIAGNOSIS	UREA	CREAT	XANTHO	INDICAN	REMARKS	RESULTS
1	Pyelonephritis	106	9.0	90	1.7	Uremia	Death-uremia, no autopsy
2	Ca of cervix, asc. pyeloneph.	148	12.8	133	Tr.	Comatose	Autopsy—Ca of cervix, pyelone- phritis, uremia
3	Ch. glom. neph.	193	20.3	142	2.0	Drowsy, musc. twitch	Death—uremia, no autopsy
4	Arteriosclerosis, acute nephri- tis:						
	2/27	83		136	1.5	Vomiting, headache	Autopsy-atrophy of kidneys, combined
	2/29	109				Vomiting	arteriosclerosis
	3/4	153	1	212	1.8		and nephritis
5	Tbc. kidney, uremia	95	8.2	111	2.1	Nausea, vom- iting	Death—no autopsy
6	Hypert. prostate, pyelonephritis	129		90	2.0	Vomiting, drowsy	Autopsy-prostate, pyeloneph. uremis
7	Pyelonephritis	176		90	1.8	Drowsy	Autopsy—asc. pyelon.
8	Ch. glom. 9/14 11/16	53 64		71 100	2.5	No symptoms Vomiting	Left hosp, and died at home, no au- topsy
9	Malig. hyperten.	123	13.0	83	3.5	Comatose, uremic frost	Autopsy—Malig nephroscler. uremia
10	Hypert. prostate, hydronephro- sis	134	9.0	100	3.0	Comatose	Autopsy-hypert. prostate, hydro- neph., uremia
11	Malig. nephro- scler.	139		100	3.0	Vomiting, restless	Autopsy—malig. ne- phrosclerosis, uremia
12	Ca of prostate, pyelonephritis	278		222	5.8	Vomiting, twitching	Died—no autopsy
13	Arteriolar ne- phrosclerosis	158		125	4.0	Comatose	Autopsy—arterio- sclerotic kidneys, uremia
14	Ch. glom. nephritis 3/30	165		83		restless	Autopsy-decomp. arteriolar-nephro.,
	4/3	216		125	2.7	Vomiting, restless	uremia
15	Arteriolar-ne- phrosclerosis	98		111	1.6	Restless, irra- tional	Died in uremia. No autopsy

TABLE 2-Concluded

CASE	CLINICAL DIAGNOSIS	UREA	CREAT	XANTHO	INDICAN	REMARKS	RESULTS	
16	Malig. nephro- sclerosis 4/12	64		47	Tr.	No symptoms		
	4/24	18		30	Tr.	No symptoms		
	5/7	66		66	Tr.	Headache		
	5/29	148		62	0.6	No symptoms		
	6/13	167		90	2.7	Sev. headache		
	6/30	203	31.6	144	2.9	Nausea, vom- iting	Died-uremia, autopsy	no
17	Malig. hypertension	204		83	1.9	Nausea, vom- iting, coma- tose	Autopsy—malig nephrosclerosis	
18	Ch. glom. nephritis	244	14.1	153	0.8	Nausea, vom- iting, coma	Died-uremia, autopsy	no

by our technic) indicates a true uremia, dependent upon retention of aromatic products, regardless of the type of pathologic lesions which produce the renal insufficiency and prognosticates a fatal termination. For in our series, all cases with high values for the xanthoproteic reaction exhibited clinical and laboratory evidence of uremia. Further, in each of these cases death occurred and was clinically attributable to renal insufficiency and uremia. In 10 cases which came to autopsy, the causes of renal insufficiency included chronic glomerulo-nephritis, decompensated arterio- and arteriolar nephrosclerosis, malignant nephrosclerosis of Fahr, obstructive (stricture, prostate, carcinoma of the cervix) pyelonephropathies, and tuberculous pyelonephritis.

In a case where symptoms of uremia are present, but where the xanthoproteic reaction is low, this type of reaction can be used to diagnose a specific pathologic lesion in the kidney responsible for the uremia. Further in such a case, the low xanthoproteic reaction does not preclude a serious prognosis. A 66 year old negro (case 17, table 1) suffered from pernicious anemia and ascending cholangitis with liver abscess. Almost concomitantly he developed restlessness, drowsiness, coma and a urea nitrogen of 173 mgm. per cent; death supervened. In view of a relatively low xanthoproteic reaction (50—which in its

slight increase above normal might have been reflected liver damage) the kidney lesion bringing about the uremia was postulated to be an acute nephritis. The post-mortem diagnosis was acute—subacute diffuse glomerulo-nephritis. A case of this type supports the experience of Becher⁴ and Rasmussen. The former explains the low xanthoproteic reaction in uremia of acute nephritis on the fact that the phenol is excreted in the urine in combination with urochromogen, whereas in uremia resulting from chronic nephritides, the urochromogen is not excreted and the phenol therefore accumulates in the blood stream.

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With the exclusion of the rôle of the xanthoproteic reaction in acute nephritis, we can say that of different chemical tests, the blood urea nitrogen in particular, the xanthoproteic reaction appears to offer the best index of uremic intoxication. Uremic symptoms in individual cases may not parallel the retention of urea nitrogen, but the retention of aromatic substances.

For instance in case 16, table 2, with urea nitrogen retention of 148 mgm. per cent and with no uremic symptoms, the xanthoproteic reaction was 62. On the other hand in case 4, table 2, with a urea nitrogen of 83 mgm. per cent uremic symptoms were prominent; the xanthoproteic reaction was 136. Similarly in case 8, table 2, with a urea nitrogen of 64, there was vomiting; the xanthoproteic reaction read 100.

The fact that the xanthoproteic reaction is a more reliable criterion of the extent of kidney damage with retention of toxic aromatic products than the urea nitrogen, gives it an important use in cases of known renal damage with moderately increased urea nitrogen but without, or with symptoms which might be interpreted as pointing to uremia. In patients 15, 16, and 18 (table 1), the urea nitrogen was elevated to 34 to 52 mgm. per cent. The xanthoproteic reading however, was normal in every case. Clinically there were no symptoms of uremia. Two of these patients were discharged improved, one died of pneumonia. Similarly in 2 cases of obstruction and ascending infection of the urinary tract (cases 2 to 4, table 1), a low xanthoproteic reaction excluded uremia and argued for a favorable prognosis, when the urea nitrogen was increased to 88 to 81 mgm. per cent respec-

tively; both patients were discharged improved following operation or therapy. In case 21 (table 1) the clinico-pathological diagnosis was tabes dorsalis, cord bladder, and ascending pyelonephritis. Although the urea nitrogen reached 81 mgm. per cent and the patient took a turn for the worse, uremia was excluded by a xanthoproteic reaction of 51. Autopsy proved that the terminal picture was caused by lobar pneumonia. There were no anatomic findings indicative of uremia.

By the same token as in the above case, a low xanthoproteic reaction supplies reassuring information in instances without kidney involvement but with an elevated urea nitrogen and with symptoms that may be confused with those of uremia, i.e., dehydration (case 14, table 1), and massive hemorrhage into the gastro-intestinal tract (case 8, table 1).

Of special significance is the differentiation between true and pseudo-uremia which can be made by the use of the xanthoproteic reaction. In pseudo-uremia, or hypertensive encephalopathy, the symptoms are due to cerebral edema and the xanthoproteic reaction remains low regardless of the urea nitrogen.

A series of repeated xanthoproteic reactions in cases with progressively failing or improving renal function, can act as a measurement of prognosis. In case 16 (table 2) the xanthoproteic reaction gradually rose, portending the appearance of uremia and the fatal termination.

In our study, we were unable to obtain information in regard to Harrison's⁶ belief, that in uremia phenols produce manifestations of depression, weakness, stupor and coma; in contrast to the manifestations of stimulation, twitching, stertorous breathing and convulsions, brought about by a calcium ion deficit. Incidently we also confirmed the findings of Steen that aromatic substances were present in the blood upon standing, and also in post mortem specimens.

Indican determinations, for all practical purposes, paralleled the xanthoproteic reaction in uremia. The lack of agreement however, in isolated cases, suggests that the two tests should be done simultaneously, or if only one is to be carried out, the xanthoproteic reaction should be preferred.

SUMMARY

1. Forty-six xanthoproteic reactions and blood indican determinations were carried out on 39 patients.

2. In the blood indican determination, we devised a new modification of the quantitative method in an attempt to reduce the percentage of error by using a definite aliquot of filtrate.

3. Except in acute nephritis, the xanthoproteic reaction gives definite information in regard to the diagnosis and prognosis of renal insufficiency, and runs parallel with the degree of uremic intoxication.

4. The xanthoproteic reaction is a simple, inexpensive, and rapid method for the diagnosis and prognosis of renal insufficiency.

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AIR EMBOLISM AS A COMPLICATION OF ARTIFICIAL PNEUMOTHORAX*

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Since the introduction of artificial pneumothorax in the treatment of pulmonary tuberculosis occasional cases of air embolism complicating this form of therapy have been reported in the literature. Bruns¹ reported thirteen cases of air embolism with four deaths, occurring during a nine year period in approximately 12.700 pneumothorax treatments. Rever and Kohl² noted ten cases with five deaths in a "series of many thousand insufflations during a period of about four years." The latter observers believe that many of those cases of pleural shock reported in the literature are, in reality, mild cases of cerebral embolism especially since the sign and symptoms in both conditions are very similar. It is quite probable that this complication is more frequent than is generally recognized. This may be due to the fact that if a fatal termination does not occur, the condition is overlooked or called pleural shock or else this type or case is not reported in the literature. The infrequency of necropsies on cases dving from air embolism and the definite proof of the cause of death warrants the recording of this case.

REPORT OF CASE

Clinical history. J. R., a white male, aged 46, was admitted to the hospital on October 20th, 1937, with symptoms of cough, loss of weight and fever. About ten years ago he had a similar symptomatology at which time an X-ray plate revealed "scars" in his lungs. The patient, however, seemed to get along fairly well until one year ago when he began to cough again and noticed that he had some fever. Four months before admission he developed some hoarseness and for the last two months was somewhat dyspneic. The patient gave no history of any tuberculous contacts.

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Physical examination revealed a well developed and well nourished white male, about 45 years of age, who did not appear acutely ill. The blood pressure was 138/90, the temperature 99.6°F., the pulse 90, and the respirations 26. The pupils were equal and reacted to light and accommodation. The oral hygiene was poor. The neck showed some enlarged submaxillary lymph nodes. The chest lagged slightly on the left side. Crepitant rales were heard in both apices. The heart was not enlarged, no murmurs were heard and the rate and rhythm were normal. The abdomen revealed no abnormalities and the genital organs and reflexes were normal. The urine examination was normal. The sputum was positive for acid fast bacilli. X-ray examination on October 20th, 1937, revealed some mottling in the upper third of the right lung. The left lung showed infiltration throughout with several cavities in the upper half of the field. There was some thickening of the pleura on this side with obliteration of the costophrenic angle. Repeated X-rays showed no change in the picture. The patient was treated symptomatically but his condition did not improve. He ran a low grade temperature and gradually became weaker. An attempt was made to collapse his left lung by artificial pneumothorax. refills were given as follows:

DATE		PRESSURES							
PALE	Start	Finish	Air						
T. 40 4000			cc.						
Feb. 10, 1938		+3 +4	150						
Feb. 11, 1938	-1 0	0 +4	75						
Feb. 13, 1938	+1 -1	+2 +4	75						
Feb. 15, 1938	-1 0	0 +1	150						
Feb. 24, 1938	-5 -1	+2 +4	200						
Mar. 3, 1938	-1 0	+2 +3	100						

He had no complaints on any refills previous to the last one. No blood was seen in the needle at any insufflation.

At the last refill just as the needle was withdrawn, the patient's face and neck became cyanotic and marked air-hunger was noted. Eyeground examination revealed air bubbles in the retinal vessels. In spite of treatment with intracardiac adrenalin, he died within a few minutes.

Autopsy. The body was that of a well developed and well nourished, white male, approximately 45 years of age, weighing about 170 pounds and measuring 172 cm. in length. No post mortem rigidity was noted and body heat was present. There was marked cyanosis of the face and neck. The pupils were equal and measured 4 mm. in diameter. There were no discharges in the ears, nose or mouth. The chest was of the emphysematous type. A needle-puncture at the site of the last air insufflation was noted on the left posterior axillary line in the fifth interspace. Another needle puncture through which the intracardiac adrenalin was administered was seen in the fourth interspace just to the left of

the sternum. The abdomen and extremities were normal. The peritoneum and pericardium were smooth and showed no abnormalities. The right pleural cavity was almost completely obliterated by fibrous adhesions except for a small area over the lower lobe anteriorly. The left pleural cavity was completely obliterated by fibrous adhesions. The pleurae were thickened throughout. No evidence of pneumothorax was found. The point of entrance of the pneumothorax needle into the lung could not be ascertained. The heart weighed 400 grams and the chambers were all dilated. A needle puncture, due to the needle used for giving intracardiac adrenalin, was noted entering the right ventricle through its anterior surface. The myocardium was reddish brown in color and somewhat decreased in consistency. The right auricle, ventricle and pulmonary artery were filled with some clotted and fluid blood. The left auricle and ventricle were dilated and filled with dark red and extremely frothy blood. The coronary arteries also contained a small number of air bubbles. The right lung weighed 700 grams and the left 800 grams. The right upper lobe was firm in consistency and its cut surface showed considerable fibrosis and many small caseous flat areas measuring from 3 mm. to 1 cm. in diameter. The lower and middle lobes were somewhat emphysematous and occasional miliary and submiliary elevated gray nodules could be seen. The left lung revealed a thickened pleura. The left upper lobe was small, firm and contracted. The cut surface showed considerable fibrous tissue with a thick walled cavity in its center measuring 5 cm. in diameter. Numerous flat yellow caseous areas were noted varying in size from 4 mm. to 1.5 cm. in diameter. The left lower lobe contained occasional miliary, submiliary, and acinous-sized elevated gray areas surrounded by parenchyma showing moderate congestion. The tracheobronchial lymph nodes were enlarged, firm and grayish black in color. The brain weighed 1425 grams. The dura was grossly normal. The blood vessels over the convex surface of the cerebri were almost completely filled with numerous large air bubbles. On lifting the brain, it was noted that the basilar artery, carotid arteries and many of their branches contained numerous large air bubbles. No hemorrhages were noted on the external or cut surfaces of the brain. The cut surfaces showed congested blood vessels. The ventricles of the brain were filled with clear cerebrospinal fluid. The spleen, liver, pancreas, gastrointestinal tract, adrenals, kidneys, and genital organs showed moderate congestion. The aorta was elastic and occasional atherosclerotic plaques were seen on the intimal surface.

Microscopic findings. The lungs revealed extensive tuberculous involvement. Section from all the other organs showed congestion but no other pathological changes were noted.

At autopsy, no pneumothorax was found although five refills had been given which, however, were of small amounts. The pleural cavity was entirely obliterated and one might postulate that the air was administered into the air spaces within the lung. Barnwell³ states that this must be the case in many inductions. This would be suggested by the manometric pressures obtained which were of a low negative or atmospheric pressure. The positive pressures obtained at the finish of the insufflations may have been due to some increased intrapulmonary pressure such as that following cough or forced expiration. In the last and fatal insufflation the air was most likely administered into one of the pulmonary veins thus causing air embolism. The pressures found at the start of the refill tend to strengthen this conclusion.

Air embolism does not occur in a normal lung because it is elastic and the blood vessels are pushed aside by the needle or retract or collapse when punctured. A diseased lung or pleura is necessary in the pathogenesis of this condition. When the lung is fibrotic and diseased, and this is the type of case in which air embolism usually occurs, the vessels are held stationary and are prevented from retracting or collapsing. An additional important factor is the increased air pressure over the pulmonary venous pressure which relationship is normally present. The perforated vessel may be located in the lung or in adhesions.

The quantity of air which will give symptoms of embolism is variable. Rabbits will die quickly when 10 cc. of air is injected into the ear veins. Nordland and his co-workers⁴ found that 30 cc. of air could be injected into dogs without causing symptoms. Amounts greater than the above caused changes in blood pressure with cardiac and respiratory irregularities. Quantities as high as 160 cc. of air usually cause death in the dog.

Air introduced into a pulmonary vein or a blood vessel within adhesions, which establishes anastomoses with the pulmonary circuit, will travel to the left side of the heart and then into the general arterial circulation. This air may come from the pneumothorax apparatus, the tubing of the manometer, the pleural space, if a partial pneumothorax is present, from an alveolus or bronchiole, the bevel of the needle connecting the air sac with the blood vessels, or from a cavity during a coughing attack if the vessels of the cavity are perforated as a result of erosion or hemorrhage.

There are three main theories as to the cause of death from air embolism. They are cerebral, respiratory with suffocation from the obstruction of the pulmonary artery and cardiac due to the lowering of intracardiac pressure and circulatory failure.

The clinical symptoms will depend upon that part of the body within which the air embolus lodges. In most cases giddiness, faintness, tickling of the throat, queer feelings, or the coughing up of blood will be complained of by the patient. The individual may become pale, go into coma and occasionally sudden death will occur. Splotching of the skin, transient blindness, diplopia, nystagmus, cyanosis, shock, profuse perspiration, chills, cardiac irregularity and respiratory failure may also be noted. The diagnosis is not always easy. One must be on the alert for a possible concomitant cerebral vascular accident simulating air embolism, functional disturbances such as hysteria, pleural shock, or cerebral embolus secondary to bronchiectases or pulmonary abscess if the pneumothorax is being given for the latter diseases.

The question of pleural shock brings up a disputed point. This condition refers to a syndrome caused by irritation or puncture of the pleura. The clinical picture produced is similar to that of air embolism. Some observers do not believe that such Others, however, bring forward clinical and exa state exists. perimental evidence for their belief in this condition. Schlaepfer⁵ failed to get any circulatory reflexes when the pleurae of normal animals were irritated and concluded that the shock seen in man did not originate in the pleura. Capps and Lewis⁶ obtained similar results, but when their experiments were repeated on dogs in whom pleuritis had been induced, they observed a circulatory response similar to that in man. Capps stresses the point that air embolism is found in few necropsies following artificial pneumothorax and states further that such a complication should take place more frequently during this procedure if it occurs but yet is quite uncommon. He notes that pleural shock is encountered most commonly in simple thoracentesis when little or no air is injected into the cavity and blames this on the fact that the exploration is usually done in the presence of acute pleuritis.

He states further that in chronic pulmonary tuberculosis, patients treated by pneumothorax do not have acutely inflammed pleurae and therefore pleural shock is less likely to occur.

The physiologic basis of pleural shock is established, according to Capps, by the analogous vasovagal reflex which can be induced in sensitive people by pressure on the carotid sinus. The symptoms arising from the carotid sinus and pleura are practically the same; namely, fainting, slow pulse, fall in blood pressure, unconsciousness and convulsions. He states that the mechanism in each is vagatonia with slowing of the pulse, and sympathetic with lowering of the blood pressure. This observer concludes that a true pleural reflex is the explanation for the great majority of accidents during operative procedures on the pleural cavities.

SUMMARY

A case of air embolism following artificial pneumothorax with necropsy findings is herein reported.

Air emboli were found in the left heart, coronary arteries and cerebral vessels, and clinically were noted also in the retinal vessels.

Air embolism complicating artificial pneumothorax may be a more common condition than is generally recognized.

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UTERINE LESIONS ASSOCIATED WITH FIBROMYOMA*

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Fibromyoma of the uterus is recognized as one of the commonest lesions of the female genital organs. Statistics based upon large numbers of cases all agree that this lesion is encountered most often during the latter part of the reproductive period. It is possible, of course, that some of the tumors arise much earlier and reach such a size as to be noted clinically only after years of slow, symptomless growth, but it seems more likely that most of them arise during the period of life just referred to, and become large enough to permit recognition clinically in a relatively few years. The age distribution, based upon the age at the time of hysterectomy, for the one hundred cases comprising this report, is shown in Figure 1. The average here is 41.7 years.

That these tumors may be found in different relationships to the mucosa and serosa of the uterus, and that their size and number vary widely from case to case, is well known. Their microscopic structure is relatively simple and constant, and has been exhaustively studied. Necrosis, hemorrhage, calcification and sarcomatous change are not particularly common, considering the frequency of fibromyomas, and, with the exception of sarcomatous change, are usually of no great clinical importance. The frequency with which sarcoma arises in fibromyomas seems often to be overestimated. In large reported series, it has occurred in somewhat less than one per cent of the cases. The detection of only one such sarcoma in the present one hundred cases is in keeping with that figure. Adenocarcinoma primary in the endometrium of the body of the uterus is no more frequent in fibromyoma cases than in uteri from patients of the same age group who do not have fibromyomas. It was found in only one of the present one hundred cases.

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The etiology of uterine fibromyoma is of considerable interest, and seems to be somewhat more amenable to investigation than

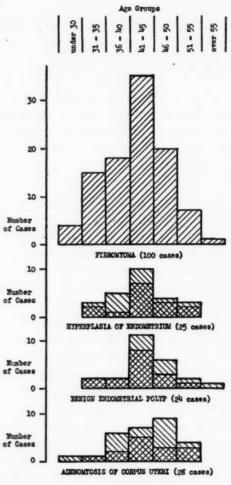


Fig. 1. Age Incidence (at Time of Hysterectomy) of Fibromyoma and of Other Lesions Observed during Same Period of Time

Cross-hatched areas in three lower diagrams represent cases also having fibromyoma. Only cases in which entire body of uterus could be examined in laboratory are included.

that of most other neoplasms. Witherspoon^{1, 2, 3, 4} has studied this aspect of the problem recently, and feels that these tumors

arise as a result of the presence of excessive amounts of estrin, as hyperplasia of the endometrium is thought to do. The present series of cases is presented partly as a commentary upon Witherspoon's work. It is hoped that these data will also serve to emphasize the frequency with which other lesions of the uterus are associated with fibromyoma. Such lesions, which will be discussed below, usually cause symptoms when occurring alone. It appears likely that, in many cases in which they are present along with fibromyoma, they, rather than the fibromyoma, are the true sources of the patients' symptoms.

The data here presented have been secured from one hundred consecutive cases studied in this laboratory, all but a few of which were operated upon in St. John's Hospital. Only three of the patients were of the colored race. In every case there was available for pathological examination the entire, intact body of the uterus. The cervix and adnexae were usually not removed, and lesions of these structures are, consequently, not included in the discussion. Every case showing one or more grossly detectable tumors, proven microscopically to be fibromyomas, is included. In a few of the cases, the only tumors present were so small (diameters as little as 2 mm) as to have been quite imperceptible on clinical examination. The largest single fibromyoma had a diameter of 15 cm.

Of the one hundred cases, it was found that forty-two showed, besides fibromyoma, one or more of the lesions mentioned below. These lesions,—the only ones observed with sufficient frequency to be of significance in a series of this size,—are hyperplasia of the endometrium, benign endometrial polyp and adenomyosis (endometriosis) of the body of the uterus. The frequency of each is shown in table 1.

The discrepancy between the sum of the figures in the table and that given above results from the fact that in some instances more than one additional lesion was observed. No case showed all three of these conditions in addition to fibromyoma.

During the same period (seventeen months) in which the one hundred fibromyoma cases accumulated, there were seen, of course, cases of the other conditions under discussion, not associated with fibromyomas. In table 2 these are listed, there

being included there only instances in which the whole body of the uterus could be examined in the laboratory. There were, as would be expected, many other cases of hyperplasia of the endometrium and of polyp, represented only by curettings. They are not included in table 2, since the presence or absence of fibromyoma could not be determined.

The diagnosis of the lesions just mentioned was made on what was considered a conservative basis. That of endometrial polyps can be open to least dispute, since they are readily noted with the naked eye. A diagnosis of hyperplasia of the endometrium

TABLE 1
LESIONS OF BODY OF UTERUS ASSOCIATED WITH FIBROMYOMA

Total cases of fibromyoma	100
Cases also showing hyperplasia of endometrium	
Cases also showing benign endometrial polyp	16
Cases also showing adenomyosis of body of uterus	14

TABLE 2

Cases of Associated Lesions Observed during Same Period as 100

Fibromyoma Cases*

LESION	TOTAL CASES	CASES ALSO SHOWING FIBROMYOMA
Hyperplasia of endometrium	25	17
Benign endometrial polyp	24	16
Adenomyosis of body of uterus	28	14

^{*} Entire body of uterus available for examination in each case.

was made only when there were found, in a thick endometrium, round or oval glands exhibiting considerable variation in size, distributed through an abundant, compact stroma. Adenomyosis was diagnosed only in cases showing patches of endometrial tissue embedded to a depth of at least several millimeters in the myometrium, and appearing, in single sections, to be quite isolated from the endometrium. Under this heading are included both more or less diffuse changes of this sort and distinct, grossly evident tumor nodules. The lesion here referred to is adenomyosis uteri interna; serosal deposits of endometrioid tissue are excluded. A good many other fibromyoma cases showed what

might be termed tendencies toward hyperplasia of the endometrium or adenomyosis, but they are not listed as such in this report.

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An examination of the average age incidence in these various conditions indicates that all of them have the same general incidence as fibromyoma. Table 3 is constructed from the cases already mentioned. Average ages at the time of hysterectomy, here and elsewhere, are given to the nearest year.

When fibromyoma was associated with one or more of the other lesions, the age incidence tended, in general, to be a trifle higher than for any one alone. The difference is so slight, however, and the number of such cases so small, that this observation, while of interest, cannot be stressed.

TABLE 3

Average Age Incidence (at Time of Hysterectomy) of Fibromyoma and Other Lesions

LESION	NUMBER OF CASES	AVERAGE AGE
Fibromyoma	100	42
Hyperplasia of endometrium	25	42
Benign endometrial polyp	24	44
Adenomyosis of body of uterus	28	43

The data just presented are shown graphically in figure 1, which is so drawn as to have corresponding age groups in the same vertical line.

DISCUSSION

The frequency with which the various conditions mentioned above are combined with fibromyoma, in the present series, is not so high as in some other reports. Witherspoon finds hyperplasia of the endometrium in practically all of his fibromyoma cases. Kanter, Klawans and Bauer⁵ report hyperplasia of the endometrium in 53 per cent, and adenomyosis in 52 per cent of their fibromyoma cases. The differences are probably to be explained on the basis of personal differences in the interpretation of the microscopic pictures observed. It may be pointed out that Reis, in discussing Kanter's paper, states that in his material

only 18 per cent of fibromyoma cases showed hyperplasia of the endometrium,—a figure almost identical with that reported here.

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The frequent association of the lesions under consideration,—which is striking even in the present one hundred cases,—as well as the similarity in their age incidences even when not associated with one another, points toward the existence of some common etiological factor. They possess certain similarities from a pathogenetic point of view, too, since all of them represent a benign hyperplastic process occurring in some component of the body of the uterus. Endometrial polyps may be looked upon loosely as localized endometrial hyperplasia, and adenomyosis as "ingrowing" polyps. This characterization is supported by the fact that often the polyp or adenomyoma presents the microscopic picture of endometrial hyperplasia, even when the endometrium itself does not. This group of uterine lesions might be thought of together as the premenopausal complex.

Witherspoon³ is of the opinion that the etiological factor in fibromyoma, hyperplasia of the endometrium, and adenomyosis is the estrogenic hormone. This is based largely upon the fact that hyperplasia of the endometrium is generally believed to result from an excess of that hormone, or from its unrestrained action on the endometrium, when not opposed by adequate amounts of corpus luteum hormone. These ideas are derived from the claim that hyperplasia of the endometrium is accompanied by multiple follicular cysts of the ovaries, with the absence of recent corpora lutea. Strong support for this conception of the etiology of endometrial hyperplasia is furnished by animal experiments and by the constant presence of marked endometrial hyperplasia in cases of granulosa cell tumor of the human ovary. The ovarian changes just mentioned are not found with regularity by all observers, however (Kanter et al5). In the cases here presented, too few ovaries were available for study to permit any definite statement in this connection, but even those few did not all display the changes mentioned.

It is suggested by Witherspoon⁴ that hyperestrinism first produces hyperplasia of the endometrium and later, if allowed to act over a period of time, causes the development of fibromyoma.

If this were strictly true, every fibromyoma should be accompanied by endometrial hyperplasia. Such a state of affairs is approached only in Witherspoon's own data. It does not seem likely that the type of lesion appearing in a given case is determined by the duration of action of some single etiological agent, since any one of the lesions can occur alone or in any sort of combination with the others.

It might be proposed, more generally, that some single etiological agent would produce a greater variety of lesions the longer it was permitted to act. In opposition to such a hypothesis, it may be pointed out that additional lesions were twice as common in association with fibromyomas less than 5 cm in diameter (37 cases out of 62) as in association with those more than 5 cm in diameter (11 out of 38). The cases with the larger tumors,—which had, presumably, been present for the longer periods of time,—were, in other words, less likely to show additional lesions.

The data at hand may be best interpreted by supposing that fibromyoma, hyperplasia of the endometrium, benign endometrial polyp and adenomyosis of the body of the uterus all possess a common etiological factor, probably hyperestrinism, but that each requires for its development some other, independent factor, the nature of which is, at present, obscure.

SUMMARY

There are presented one hundred cases of fibromyoma of the body of the uterus. In almost half of them, other lesions of the body of the uterus were also present. It is concluded that fibromyoma and these other lesions probably have a common etiological factor, but that other factors must participate in the production of each of them.

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EXAMINATION OF CULTURES FROM PERSONS SUS-PECTED OF HAVING CHRONIC INFECTION*

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During 15 years investigation of the significance of immunobacteriologic reactions of streptococci, staphylococci, Micrococcus catarrhalis and colon bacilli isolated from persons suspected of having chronic infection, certain observations were made which resulted in better understanding of the pathological relationships of these organisms. A summary of these findings will be presented in this paper.

The difficulty of applying Koch's postulates has been a major problem in the bacteriological study of chronic infection. This has made it necessary to seek proof of etiologic relationships by indirect methods, such as attempts to show a higher frequency of some particular group of bacteria in "infected" persons than in "controls." Because of the difficulty of determining the presence or severity of infection in certain cases, it is likely that many persons with latent or hidden infection were used as "normal" controls. As a consequence, such experiments had less significance than similar tests in diseases in which infection and freedom from infection were rather sharply differentiated.

Another diagnostic difficulty was caused by the fact that certain "immune" bodies encountered in chronic infection studies are really response bodies, but are produced irregularly and have no constant relationship to infection, immunity or sensitization. They are further complicated by cross-reactions. Eastwood¹ called attention to the fallacy of considering that the characters

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of antibodies in vivo and in vitro are the same, and pointed out that active immunity could be acquired without the production of serological antibodies. Teale² showed that an immunized animal could clear streptococci, pneumococci, Friedlander bacilli and B. anthracis from the blood in the absence of agglutinins, bactericidins and protective antibodies for the invading micro-organisms. Gilbert and Dacey³ reported the recovery of Brucella abortus from the blood clot of a patient whose serum did not agglutinate Br. abortus. These irregularities, together with the alteration of antigenic structure of the cultures as a result of dissociation, have prevented successful application of bacterio-immunologic reactions to the study of chronic infection.

A general relationship has been shown between complement fixing properties of unheated blood serum and the electrophoretic migration velocities of B. coli isolated from the feces of the person from whom the blood was obtained. However, complement fixation reactions using a wide variety of bacterial antigens with active serum were not highly specific⁵. For example, there was only 65.3 per cent agreement between the presence of pathogenic type staphylococci in cultures from a person and the staphylococcal complement fixing power of his blood serum⁵. The results with other groups of bacteria appeared to be even less specific. The frequency of cross-fixation, the failure of usual methods for titrating antigens, the degeneration of antigens, the lack of complement fixing power in certain diseases, the presence of a non-specific anticomplementary property which existed in different strengths in different specimens of blood, and the difficulty of controlling the reactions prevented successful application of the method with active serum5.

There was no relationship between agglutination reactions and certain in vitro tests which will be discussed later, except for Staphylococcus albus, in which there was agreement in 72.5 per cent of the cultures. These in vitro tests had been shown to be parallel with certain pathogenic properties of the cultures and were used to study the relation between immunologic reactions and possible pathogenicity of the cultures.

There was agreement between intradermal tests and the in vitro tests in 83 per cent of cultures of Micrococcus catarrhalis,

in 84 to 87 per cent of staphylococci, and in 70 per cent of gamma type streptococci, but there was no agreement in Streptococcus viridans, hemolytic streptococci or colon bacilli⁷.

There was agreement between intradermal tests and agglutinability in 91 per cent of enterococci, in 100 per cent of Micrococcus catarrhalis, and in 70 per cent of staphylococci, but there was

no relationship in other groups7.

Based upon the bactericidal power of the blood, Solis-Cohen and his associates developed a technic⁸ which they refer to as the "pathogen-selective" method. The assumption is made that the blood of a person with certain types of infection is able to kill organisms which have not invaded the tissues of his body, and that organisms which are able to survive are pathogenic for that individual. Although most of those who have used the method have accepted it without reservation, Solis-Cohen and Rubenstone⁹ called attention to its limitations, pointing out that the reaction was to a certain extent quantitative and was affected by the proportion of blood and bacteria.

Short, Dienes and Bauer¹⁰ discussed errors in the theoretical basis of such tests. Rawls and Chapman¹¹ reviewed the literature on the relation between resistance to the bactericidal power of the blood and certain pathogenic properties of the bacteria. Most of the evidence suggested that the "bactericidal" power of the blood was dependent upon a general antibacterial property

which was closely related to complement activity.

The parallel between the power of streptococci to survive exposure to fresh blood and exposure to certain chemicals suggests that the ability of certain bacteria to overcome the bactericidal power of the blood may depend upon the greater resistance of pathogenic strains to unfavorable environmental conditions and not upon a specific differential property of the blood. Thus, tests based upon the bactericidal power of the blood should determine pathogenicity of the micro-organism for the general population rather than for the particular person from whom the blood was obtained. Hare¹² tested organisms against normal blood and the blood of the patient and concluded that in general there was no difference. Fleming and Petrie¹³ were of the same opinion.

A further difficulty with the method lies in the high proportion

of non-pathogens which are capable of growing in the patient's own blood. Boerner and Solis-Cohen¹⁴ reported that 35.1 per cent of Staphylococcus albus, 42.8 per cent of diphtheroids, 33.3 per cent of unidentified micrococci, and 100 per cent of Proteus vulgaris grew in the patient's blood. They stated also that the method is not applicable to spore-forming organisms, to those producing exotoxins (although they used it for Staphylococcus aureus and hemolytic streptococci, both of which may produce exotoxins), or to those difficult to cultivate.

Results with mixed cultures may not be as specific as those with pure cultures, as suggested by experiments with streptococci¹⁵. Finally, because a different technic is required for each group of bacteria, it is impossible to select a technic or a dilution of blood which will be suitable for all groups.

The foregoing observations illustrate the difficulty of establishing the identity of the infectious agent in chronic infection studies. A new approach to the problem appears to lie in the study of the pathogenicity of the suspected bacteria. Micro-organisms with pathogenic properties would more probably cause chronic infection than would organisms with non-pathogenic properties, which are more likely to be degenerate daughter races, contaminants or secondary invaders. While individual differences in resistance may account for differences in susceptibility to infection, such differences should not make a person more susceptible to non-pathogens than to pathogens. It was pointed out by Chapman et al. that, in staphylococcal infections of the skin, the degree of pathogenicity of the invading micro-organisms, as indicated by in vitro tests, was parallel to the severity of the infection.

The problem of dissociation and its effect upon antigenic properties is a difficult one. Streptococci and staphylococci are easily dissociated, even in the tissues of the body. In many cases, the dissociation is accompanied by antigenic changes, with the result that the antigenic properties of the dissociated daughter races may be quite different from those of the parent strain and, unless the dissociants are separated, serologic and immunologic tests may give erroneous results. Saelhof¹⁷ isolated a streptococcus in two forms from the urine of a patient with pyelocystitis.

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The diphtheroid phase was non-pathogenic for rabbits but the streptococcal phase produced pyelocystitis in rabbits. It was shown^{16,18} that many strains of staphylococci from pathologic sources are mixtures of dissociants, some of which are highly pathogenic, while others are non-pathogenic. McGaughey¹⁹ isolated two variants from a toxigenic culture of Clostridium Welchii. The first produced small amounts of toxin after prolonged cultivation, while the second produced 3 to 6 times as much toxin as the parent culture. Pathological differences associated with smoothness and roughness are well known.

In view of these observations, a more critical analysis of cultures from the standpoint of possible differences in antigenic structure within a culture should assist in understanding the bacterio-immunologic relationships in chronic infection.

In attempting to develop simple methods for estimating probable pathogenicity, comparison was made of certain in vitro tests and animal inoculation tests of streptococci, staphylococci and colon bacilli²⁰. It was argued that, if these in vitro tests gave results reasonably parallel with animal inoculation tests, if they were subject to similar or smaller errors, if they were simpler and more easily made in duplicate, then for practical purposes, such in vitro methods should be more practicable for studying large numbers of cultures.

The animal inoculation tests had several disadvantages: They required from 2 to 5 days; several animals were necessary to test each culture; it was difficult to measure accurately the amount of culture injected; 14.7 per cent of the animals died from intercurrent infection, shock, emboli, etc.; and animals (e.g. rabbits) available from supply houses did not have the resistance possessed by those especially bred for such purposes.

In contrast, the in vitro tests had several advantages. They were easily reproducible within a small margin of error, a "loopful" was a sufficiently precise unit for measurement, and the results were obtainable within about 18 hours. For these reasons, they offered hope of being useful as substitutes for animal inoculation tests, when the latter were impracticable.

Because animal inoculation tests were used to appraise the

accuracy of the in vitro tests, any error in the animal tests reflected itself as an apparent error in the in vitro tests. However when the irregular results of animal inoculation experiments were taken into consideration, the in vitro and animal inoculation tests gave similar results. While the error of the in vitro tests differed with the particular tests, and while the precision of the results depended upon technical skill and experience, these tests were subject to fewer irregular results than animal inoculation tests and, hence, were considered more satisfactory, particularly when only one test was made on each culture. The use of two or more independent in vitro tests increased the accuracy of the tests

The test for coagulase production, supplemented by tests pigment and hemolysin, was used for staphylococci¹⁶. Resistanto injurious agents was used for streptococci^{14, 21}. Electrophoresis was used for colon bacilli⁴. Crystal violet agar was used for the Micrococcus catarrhalis group²².

These in vitro tests were applied to cultures obtained from a large series of patients. In most cases, one or more serological or immunological methods was used also. In some patients, there was a high degree of correlation between different tests. In others, the results were difficult to interpret.

For example, in M. E. C. (table 1) there was agreement between intradermal tests, agglutinability and in vitro tests of the different groups. Hemolytic streptococci and Staphylococcus aureus gave positive results with all three methods, while Streptococcus viridans gave essentially negative results with all three. In Streptococcus viridans \$1353 it was impossible to make agglutination tests because of autoagglutination, but it was possible to carry out resistance tests and intradermal tests.

R. K. (table 2), who had evidence of infection with several groups of bacteria, affords an example of the uses and limitation of certain tests. The positive complement fixation reactions (active serum) to certain groups were not constant, which suggests that the results may have been influenced by the antigens used at different times. However, Staphylococcus albus and aureus gave strongly positive complement fixation every time. This patient had ethmoiditis, and a strain of Staphylococcus

aureus which reacted positively to all 6 in vitro tests was isolated from the ethmoid secretion. At first, the electrophoretic mobility of one of the B. coli strains was P.D. 48, which was considered characteristic of a pathogenic culture, and the complement fixation reaction with B. coli antigen was strongly positive.

TABLE 1
CORRELATION OF DIFFERENT TESTS MADE ON M. E. C.

		вот	RCE		CUL- TURE NUM- BER		IN VITRO	INTRADES	INTRADERMAL TESTS	
ORGANISM	Feces	Throat	Left	Right		AGGLUTI- NATION TITEB	OF PATHO- GENIC- ITY	Imme- diate	24 hr.	
B. coli	150*					0	0			
Strep. alpha	1000*				1348					
		Few			1350	0	0	0	+	
		Mod.			1353	Rough	0	0	++	
		Mod.			1354	0	0	++	+	
			Few		1403	0 .	+	0	++++	
		Mod.			1612	0	0			
		Mod.			1613	0	0			
		Few			1614	0	0			
		Few			1616	0	0			
Strep. beta		Few			1615	1:10,240	+	+	++++	
			Few		1609	1:10,240	+	+	+++	
				Mod.	1608	1:10,240	+	++	++++	
Staph. aureus	800*				6305	1:640	+	++++	++++	
		Few			6309	1:1280		++++	++++	
			Many		6307	1:2560		++++	+++	
				Many	6308	1:2560		++++	++	

^{*} Millions per 100 grams dry feces.

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Twenty months later the P.D. had changed through 53 and 56 to 58, considered in the non-pathogenic range, and the complement fixation reactions with B. coli antigens were negative. Hemolytic colon bacilli were isolated in January 1930 and complement fixation with this group was positive in December of that year. Friedlander bacilli were recovered from the sinus secre-

TABLE 2 Comparison of Tests on R. K

COMPLEMENT FIXATION	7/11/29	1/7/30	3/25/30	12/11/30	4/7/31	11/5/31	5/8/32	4/5/38
B. coli	+++	++++		++++	++++	0	0	
A. aerogenes	++	++			++	0	0	
Citrate neg. inter	0	++++		0	++++	++	0	
Sl. hem. coli	0	++++		++	++	0	0	
Hemolytic coli	0	0		+++	0	++	0	
Salmonella	0	0						
Typhoid	++++	0		++++	++++	++++	0	
Dysentery	0	0		0	0	0	0	
Pseudomonas	0	0		0	0	0	0	
Proteus	0	0		++	0	0	0	
Alcaligenes	0	0		0	0	0	0	
Friedlander	0	0		++++	++			
Diphtheroids	0	0		0	0	++	0	
Monilia	0	0		0	0	0	0	
Cl. Welchii	0	+++		0	0	0	0	
Other anaerobes	0	0		0	0	0		
Strep. viridans	++	0		0	0	++	0	
Strep. gamma	++	+++		0	0	0	0	
Hem. strep	0	0		0	0	0	0	
Enterococcus, gamma	0	0		0	++++	0	0	
Enterococcus, alpha	0	0		0	0	0	0	
Enterococcus, beta	0	0		0	0	0	0	
	++++	++++		++++	++++	++++	++++	
Staph. aureus		++++		++++	++++		++++	
Gonococcus	0	0		0	0			
Other Neisseria	0	++++		0	0	++++	++++	
Monilia	0	0		0	0	0	0	
Hemophilus	0	++++		0	0	0	0	
B. Morgani I				++++	++++	++++	0	
Br. Abortus				0	0	0	0	
Feces:								
Cl. Welchii	40,000	4,800	600	200	3,500	(mill.)	per 100 gr	ns. dry)
B. coli (P.D.)	48	48, 69	53, 73	56, 72	58			
Hem. coli (P.D.)		50						
Aerog. (P.D.)		48						
B. Morgani I (P.D.)			55					
Sinus secretion:								
Friedlander (P.D.)					49			Present
B. coli (P.D.)					59			
Staph.(pathogenic type)			Present					Present
Pseudomonas	1			1	Present			Present
Strep. (path. type)								Present
Bile:								
Hem. coli (P.D.)			55					
Non-hem. coli (P.D.)	1		55					
Eberthella (P.D.)			56	1				
Staph	1		Present					

tion and from the feces. The electrophoretic potential of the strain from feces was P.D. 50 and of that from the sinus P.D. 49.

Because of other similarities, this suggests that the two strains were related. The complement fixation reaction with typhoid antigen was positive on most occasions and a strain of Eberthella which, however, did not give specific agglutination, was recovered from the bile. Since the electrophoretic mobility of the strain was P.D. 56, which is considered to be in the borderline range of pathogenicity, it is possible that it was a degenerate typhoid bacillus. A culture of B. Morgani I was isolated from the feces in 1930 and the complement fixation reaction to this group was strongly positive on three subsequent occasions. The presence of Clostridium Welchii in large numbers was explained by the fact that there were numerous domestic animals on the patient's estate. However, complement fixation reactions with Cl. Welchii antigens were negative, except on one occasion. The agglutination reaction with Brucella abortus was positive in a dilution of 1:50 but complement fixation reactions with this group were negative. Complement fixation reactions with streptococcus antigens were negative almost every time but strains reacting 8+ to the resistance tests were isolated on several occasions from the nose and throat. Since many patients with evidence of streptococcal infection gave negative complement fixation reactions with streptococcal antigens, it is possible that the negative results in this case were caused by failure of the invading organism to stimulate the production of complement fixing antibodies.

While the difficulty of establishing the presence or absence of infection in most cases of chronic disease makes it impossible to determine the specificity of the different reactions, it is possible from the experience gained from this investigation to draw certain general conclusions.

Each of the tests discussed has certain disadvantages. The serologic and immunologic reactions appear to depend largely upon certain pathogenic properties of the cultures. The irregular production of antibodies in many persons with chronic infection and the apparent dissimilarity between the presence of serologic antibodies and immunity to the invading micro-organisms would leave the pathogenic properties of the micro-organisms as the common denominator of the host-parasite relation

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ship. Therefore, better understanding of bacterio-immunologic reactions would seem to lie in better knowledge of the pathogenic properties of the bacteria.

A priori, this would be taken to mean that the presence of a pathogenic micro-organism in a culture from the body always indicates an infection by that organism. Obviously, such an assumption is contrary to common knowledge. However, the presence of a pathogenic micro-organism in increased numbers would have more significance than when the organisms are non-pathogenic. A number of such relationships have been shown in the monographs discussed in this paper.

The serologic and immunologic reactions discussed in this paper appear to be more useful for certain groups of bacteria, and for this reason may be of lesser value than simple tests which may be more closely related to the pathogenic properties of the cultures. In the two patients just cited, the in vitro tests which had been shown to give results parallel with certain pathogenic properties appeared to give as much information as was obtained by serologic and immunologic tests and, consequently, would be preferable on the basis of simplicity.

The evidence obtained in this investigation suggested that certain in vitro positive bacteria are capable of implanting themselves in the respiratory and gastrointestinal membranes at an early age, and that these organisms may remain constantly, although the number may vary from time to time. On several occasions in vitro positive bacteria were isolated from apparently normal areas which further investigation proved to be chronically diseased.

Progress depends upon more accurate knowledge of dissociative or degenerative tendencies and their effects upon serologic and immunologic reactions. When these have been accomplished, it should be possible to obtain better results with the different tests of bacteria isolated from possible foci of infection.

SUMMARY

An analysis of the theoretical basis for, and practical tests of, serologic and immunologic reactions which have been proposed

for determining which organisms have invaded the tissues of a particular person suspected of having chronic infection indicates that each method is subject to error. In certain instances, the tests apparently resolve themselves into crude tests of pathogenicity of the suspected bacteria.

Since the pathogenicity of the micro-organisms may be a factor in their ability to implant themselves in susceptible tissues, it is suggested that tests of pathogenicity may be of value in chronic infection studies. Because of the difficulties involved in applying animal inoculation tests, in vitro tests which give results parallel with pathogenic properties should prove useful when it is necessary to study large numbers of cultures.

Discussion of the relationships of the results of these in vitro tests suggests that further study, with particular reference to antigenic differences in dissociants, should throw light on the problem of the sero-bacteriology of chronic infection.

CONCLUSIONS

Each of the serologic, immunologic and bacteriologic methods proposed for the differentiation of bacteria thought to be the cause of certain chronic infections is subject to considerable error.

Because of the possibility that pathogenicity of the invading micro-organisms may play a major rôle in the host-parasite relationship, it is suggested that further study of the pathogenic properties of bacteria isolated from persons suspected of having chronic infection may be of value in better understanding of bacterio-immunologic reactions of such persons.

Certain in vitro tests have been shown to have been useful in appraising the significance of these organisms.

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MEDICOLEGAL ASPECTS OF CHORIONEPITHELIOMA IN THE MALE*

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Chorionepithelioma of the testis is a rare disease. Ferguson¹ found in a series of more than 400 testicular tumors, but six chorionepitheliomas. Whether primary retroperitoneal chorionepithelioma exists at all, is still a matter of controversy. Symeonidis², in a review of the literature until 1935, accepts only two such cases as authentic.

The following case is reported because it is of interest from the medicolegal standpoint.

CASE REPORT

A single, white man, 24 years of age, railroad watch inspector, entered St. Francis Hospital in April 27, 1938. He complained of a mass in the abdomen which, in his opinion, was caused by an accident. Early in March, he had lifted a railroad hand car when on inspection tour, and had immediate, intense pain in the back. On the next day, he consulted an orthopedic surgeon. X-ray pictures of the thoracic and lumbar region did not reveal any fracture or dislocation. The orthopedist made a diagnosis of sprain of the back and gave several infrared light treatments. Three weeks later, when taking a bath, the patient felt a mass in his abdomen. A surgeon was consulted who advised exploratory laparotomy.

At entrance in the hospital, the patient's general health was good. A fist-sized mass could be palpated in the abdominal cavity, in the region of the right kidney. It was tender and firmly attached to the posterior abdominal wall. A pyelogram revealed a low right kidney. The calices appeared flattened out, probably from pressure of the tumor. The preoperative diagnosis was renal or adrenal tumor.

Operation. May 5th, an exploratory laparotomy revealed a retroperitoneal tumor which was soft, hemorrhagic and partly cystic. It was attached to the lumbar vertebrae and was not connected with the right kidney. Two pieces

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of fragile tissue were removed for histologic study. They were hemorrhagic and in small parts of yellow color, and measured 12 x 15 and 8 x 30 mm. respectively.

Microscopic findings. Paraffin sections stained with Hematoxylin and Eosin revealed large hemorrhagic and necrotic areas and only few well stained portions. There were two types of tumor cells noticed. Placques of cells with definite outlines and vacuolated light stained protoplasm were predominant. The nuclei of these cells were vesicular and had one or two coarse nucleoli. The strands of these cells were surrounded by syncytial cells with deeply staining

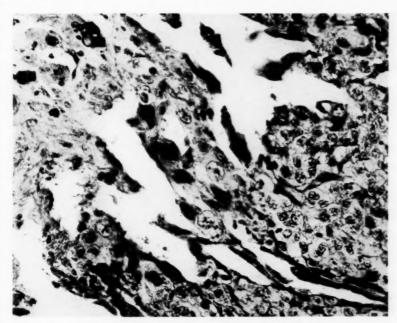


Fig. 1. Biopsy Specimen from Retroperitoneal Tumor Plaques of Langhans' cells and syncytial cells. $(\times 240)$

cytoplasm and multiple hyperchromatic nuclei. These syncytial elements often were lining large spaces filled with red blood cells.

The pathological diagnosis was chorionepithelioma and the clinician examined the patient carefully for a primary testicular tumor. Both testes were small and there was no palpable mass found. Hormonal assay of the urine, following the technic of Cutler and Owen, 3 revealed 5,000 mouse units of prolan B.

Six weeks after operation, the patient developed cough and pain in the chest. The X-ray picture showed extensive nodules throughout both lungs. There was a pneumothorax at the right side and a large effusion in the left pleural

cavity. The heart shadow was obscured by dense masses. The patient's condition became worse from day to day and he died on June 23, 1938, sixteen weeks after the injury.

Necropsy. The pathologist being absent, a house physician performed the necropsy. In the right pleural cavity about 1,000 cc. of bloody fluid was found, in the left 500 cc. Both lungs were studded with large circumscribed nodules varying in size from 0.5 to 3 cm. in diameter. On section, the nodules were extending throughout the lungs which were deeply congested. The cut nodules were grayish pink with bloody areas. The liver was not enlarged. The surface

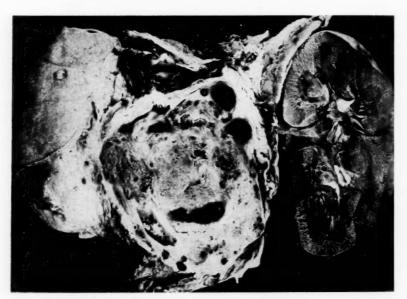


Fig. 2. Retroperitoneal Tumor. In Right Lower Portion Invasion of Inferior Vena Cava

was smooth and no tumors were visible. The cut surface of the right lobe was studded with many hemorrhagic round nodules measuring between 1 and 2 cm. in diameter. Many bile ducts were markedly dilated. Very few nodules were present in the left lobe.

Retroperitoneally, a mass measuring 6 x 8 cm., was firmly adherent to the posterior abdominal wall between both kidneys. It extended from the first to the third lumbar vertebra. The large abdominal blood vessels were completely surrounded and the inferior vena cava was invaded by tumor tissue and contained a soft grayish mass, 1.5 cm. long. The cut surface of the retroperitoneal tumor was mottled and was composed of grayish, hemorrhagic and yellowish

areas. The kidneys were not invaded by this tumor and were of normal gross appearance.

The right testis measured 3.5 x 2 cm. and the left 3 x 2.3 cm. Both were of soft consistency and apparently without any tumor.

It was concluded that this was a case of extragenital chorionepithelioma and that the injury was an important factor in aggravating, if not in precipitating, the development of the growth.

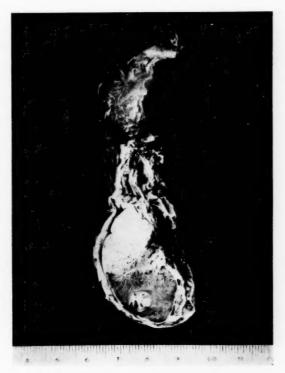


FIG. 3. TERATOMA IN RIGHT TESTIS

The tumor in the retroperitoneal region, the nodules in liver and lungs presented the same histological picture as did the biopsy specimen. In all specimens, Langhans cells and syncytial cells were contained in varying proportions. There was widespread hemorrhage and necrosis. No teratomatous adult structures were found in the retroperitoneal tumor. The testes, which were smaller than normal, were dissected in 2 mm. thick sections. In the right testis, a well circumscribed oval nodule was located, near the lower pole. It measured 7 x 9 mm. It was soft, of grayish color and contained several small cysts. Micro-

scopic sections revealed islands of hyaline cartilage, nerve and unstriated muscle and spaces lined by columnar and stratified squamous epithelium. Near the periphery of the teratoma, anastomosing strands of darkly staining round cells were found. These cells had scanty protoplasm and vesicular nuclei with large nucleoli. These sheets of indifferent cells resembled the description of immature Langhans cells recorded by Teacher⁴ in connection with uterine chorionepithelioma. Syncytial elements were not evident. Only in the wall of a small cyst several large acidophilic cells with multiple nuclei were protruding into the

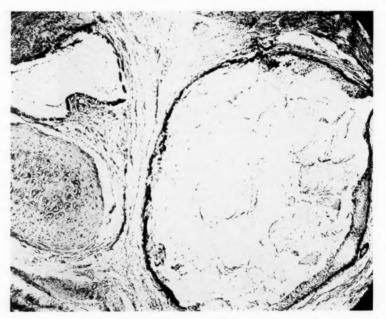


Fig. 4. Teratoma of Testis

Cysts lined with columnar and squamous epithelium. Island of hyaline cartilage. $(\times 70)$

lumen. There was a marked hypertrophy of the interstitial cells in the surrounding testicular tissue. It resulted in strands and nodules up to 500 microns in diameter. This proliferation of Leydig cells has been reported also by Arendt⁵ and Heaney.⁶

The statistics of Greiling⁷ compiled from a study of 220 cases of malignant tumors of the testis with metastases show retroperitoneal involvement in 100 per cent of the cases. Such figures

justify one in doubting the validity of the reported cases of primary retroperitoneal chorionepithelioma with no or incomplete examination of both testes. Prym⁸, in reviewing the literature until 1930, states that the reported primary retroperitoneal chorionepitheliomas were in reality metastases of overlooked testicular teratomas. According to Symeonidis² only Miller's and Browne's case and the case of Fenster appear to fulfill the rigid criteria necessary for the diagnosis of primary retroperitoneal chorionepithelioma. The primary tumor may be so small that it evades not only examination before death, but also routine autopsy procedure. The primary tumor may be hemorrhagic or necrotic, it may be consisting only of a few adult teratomatous elements, or it may have healed spontaneously as in Prvm's⁸ case. While in this case the metastatic nature of the retroperitoneal chorionepithelioma has been established, there is the question whether the injury must be regarded as responsible for the unusually rapid course of the disease. According to Belt¹¹ chorionepitheliomas show wide variations in rapidity of growth and duration of the disease. The outcome is uniformly fatal. Craver and Stewart¹² described a case of chorionepithelioma in a 15 year old boy where both testicles were atrophic. The only complaint was dyspnoea and pain in the chest. Death occurred in three months from general metastases. Also, without injury, chorionepithelioma in the male may be just as short in duration as in my case. Trauma is to be accepted as an aggravation only if death of the patient is hastened definitely.

There is another question, whether an injury can cause appearance or localization of metastases. Many metastases are, in fact, referred by patients to previous trauma. Ewing¹³ believes that the possibility of the localization of a metastasis by a trauma may not be excluded. He reports a case of malignant testicular tumor with epigastric metastases. The patient was given several hypodermic injections in the deltoid region. Two weeks after the injections, a nodular swelling developed in this area which had the structure of the testicular growth. In Gottlieb's¹⁴ case, a tumor of the eye developed following a head injury. The eye was removed and microscopic examination revealed a metastatic

chorionepithelioma. The patient claimed that the tumor was caused by the head injury and sought compensation.

Malignant testicular tumors metastasize most frequently in the retroperitoneum, as shown by the statistics of Greiling. Since the development of retroperitoneal metastases belongs to the typical picture of chroionepithelioma, the trauma which this patient suffered, cannot be accepted as responsible for appearance or localization of the metastatic growth. The rapid course of the disease following detection of the retroperitoneal tumor is explained by the invasion of the inferior vena cava, an occurrence which is also typical for chorionepithelioma and entirely independent of the trauma.

CONCLUSIONS

In a 24 year old man, a retroperitoneal tumor appeared three weeks after an injury to the back. Histological examination of a biopsy specimen revealed chorionepithelioma. No primary tumor was found by clinical examination in the testes. In the urine, 5,000 Mouse Units of Prolan B were found. The patient died 16 weeks after the injury with metastases in liver and lungs.

By serial sections of both testes, a small teratoma was discovered in the right testis. It consisted mostly of adult elements and very few non-differentiated cells.

Compensation under the workman's compensation act was sought. From the autopsy findings, it was extremely unlikely that the injury was responsible for the appearance of the retroperitoneal tumor or for the unusually rapid course of the disease.

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SPECIFICITY IN THE SERODIAGNOSIS OF SYPHILIS*

A DIFFERENTIAL METHOD

PRELIMINARY REPORT

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It has long been recognized that various bacterial diseases other than syphilis, as well as certain noninfectious conditions, may give rise to positive serologic reactions and it has been assumed that heterophile manifestations and protein disturbances are actual causes of nonspecific serologic reactions. "Wassermann-fastness" can perhaps be explained on the same basis, although in some instances other factors most likely are of significance.

In the present work, the bulk of protein was removed, by means of copper sulphate, from the serums to be tested while, apparently, the syphilitic antibody or reacting substance remained in solution together with only traces of protein.

Removal of the serum protein

The majority of the blood samples had been stored at ice box temperature for 10 to 12 hours and finally left at room temperature for several hours before starting the procedure. The serums used were all clear and free of hemolysis. Into the respective tubes was measured 0.15 cc. of serum from the various samples showing positive serologic reactions by several different methods (in the majority of cases by Rytz, Kahn, Kline, Mpls. Gen'l. Hosp. Wassermann, and Kolmer Wassermann). The serums were then heated in a water bath at about 55°C. for 5 minutes. After cooling at room temperature, 0.3 cc. of 2 per cent copper sulphate (Mallinckrodt) in distilled water was added to each tube and the rack shaken for a few seconds. Then 0.5 cc. of distilled water was measured into each tube, and, one by one, the tubes were tilted 2 to 3 times to mix the contents. All tubes were then centrifuged at about 2,000 revolutions

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per minute for 5 minutes and, showing a water clear supernatant fluid with the protein at the bottom, again placed in the rack.

Serologic technic

From each tube was transferred 0.15 cc. of clear supernatant fluid into other tubes measuring approximately 9 mm. (inside diameter) by 75 mm. To each tube was then added 0.05 cc. of half saturated ammonium sulphate (1 part of unheated saturated solution to 1 part of distilled water), and the contents were mixed by shaking the rack for a few seconds before the addition to each tube of 0.03 cc. of antigen emulsion as described below. The contents were again mixed by shaking the rack for a few seconds. All tubes were then centrifuged at about 2,000 revolutions per minute for 5 minutes. After centrifugation, 2 cc.



Fig. 1. Inverted Fisher Kahn Lamp for Convenient Reading of This Reaction

of distilled water were added to each tube, and all tubes were slowly inverted twice and again centrifuged at about 2,000 revolutions per minute for 5 minutes. The fluid content was decanted by carefully inverting a handful of tubes, allowing drainage to the last drop before placing them in normal position in the rack. To each sediment was added 1 cc. of a 1:20,000 distilled water solution of potassium oxalate (Mallinckrodt, Pure, Neutral) which was prepared each day shortly before use. Each tube was inverted slowly once, and all tubes were then left at room temperature for 10 to 15 minutes and again inverted slowly once or twice before reading the results by a lamp as shown in figure 1.

The reactions from known syphilities showed a fine but distinct flocculate, and verified nonspecific reactions (with false positive routine test) exhibited a hazy fluid without flocculate.

The antigen employed

Rytz antigen H¹ was employed in this procedure. It was prepared as follows: Fifty grams of Difco beef heart powder were placed in 300 cc. of anesthesia

ether, contained in a 1 liter Erlenmeyer flask, and shaken for 5 seconds every 10 minutes for one hour. The suspension was then filtered through a dense filter paper and the powder allowed to dry completely by spreading it on a filter paper. The dried powder was again placed in an Erlenmeyer flask to which was added 300 cc. of pure acetone. This was shaken for 5 minutes and then filtered as before. To free the powder from the last drops of acetone, it was pressed lightly with a tongue blade; in order to dry completely, the powder was placed in the incubator for three hours. The dry powder was weighed and suspended in absolute ethyl alcohol in the proportion of 1 gram of powder to 5 cc. of alcohol. This suspension was shaken in a mechanical shaking apparatus for $1\frac{1}{2}$ hours and filtered immediately through a dense filter paper. The alcoholic extract was measured, and 0.6 per cent cholesterol (Merck) added and dissolved by rotating the bottle (glass stoppered) in a water bath at about 50°C. The cholesterolized extract was filtered and stored in small, brown, glass-stoppered bottles at room temperature and protected against light. It was not used for a longer period of time than 6 months after the date of preparation.

Emulsification of the antigen

Into a large test tube was measured 0.6 cc. of 0.45 per cent sodium chloride in distilled water. To that was added slowly 0.7 cc. of 1 per cent cholesterol (Merck) in absolute ethyl alcohol. This was rotated lightly for a few seconds, and 0.6 cc. of the antigen was added and the tube shaken vigorously for about 1 minute. The emulsion was diluted with 2.5 cc. of 0.9 per cent saline, the tube again shaken for 1 minute, and the contents were poured into a small serology tube and centrifuged for 5 minutes at high speed. The supernatant fluid was decanted by inverting the tube, and 2 cc. of 0.9 per cent saline added to the sediment which again was shaken into an even emulsion and heated in a water bath at about 55°C. for 5 minutes. The usual procedure was to prepare the emulsion and heat it together with the serums each day.

THE RESULTS OBTAINED

As shown in a previous publication², pooled beef serum gives positive flocculation reactions by the various routine methods. By the present procedure such serum showed negative reactions. Rabbits inoculated with flocculate obtained by routine flocculation tests from beef serum and from human syphilitic serum, have been shown to develop positive routine syphilitic reactions². Such rabbit serum, showing false positive routine reactions, gave invariably negative results by the present method. On the other hand, syphilitic rabbits, injected repeatedly with flocculate, promptly registered positive reactions also by the present procedure.

False positive routine flocculation tests were induced in negative human serums through contamination with B. Coli. To 1 cc. of normal serum was added 0.05 cc. from a broth culture of B. Coli, and 5 percent of 100 serum samples thus treated developed false positive routine flocculation tests after twenty-four hours incubation at room temperature. By the present procedure, all of these serums gave negative reactions.

Blood samples were obtained from 1000 patients with positive routine syphilitic tests, by the various methods, in the Minneapolis General Hospital and the out-patient department of that institution. Of that number, 880 were confirmed as positives by the present procedure, and the remaining 120 were found negative. A comparatively small percentage of the 120 negatives by this method were from patients with acute infections, measles. pneumonia, malaria, lead poisoning, rheumatic fever, etc., all without syphilitic history but positive by two or more of the routine tests; all of these patients gradually became negative by all methods without subjecting the patient to specific treatment. The great majority of the cases negative by the present procedure were patients without syphilitic history, apparently diagnosed "latent lues" on the strength of positive "routine" serology by the various methods and given antiluetic treatment for three years or longer without apparent change in the serologic reactions and therefore termed "Wassermann-fast."

Cases with a diagnosis of neurosyphilis have not been considered in this work.

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UNUSUAL NEOPLASMS OF THE SMALL INTESTINES*

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A review of the literature shows that the number of reported cases of neoplasms of the small intestine is considerably greater than is generally reported in the text books or by individual authors reporting such cases.

With more precise clinical and roentgenological methods of diagnosis, it seems advisable to become more familiar with the types of tumors encountered and their location.

The number of cases of malignant tumors reported is greater than the number of benign tumors. Carcinoma is more common than sarcoma. Next to lymphosarcoma, myosarcoma is more common than the other types. In some series of carcinoma of the small intestine the duodenum is given as the most common location, while in other series the ileum is the most common site of the tumor.

Nothnagel between 1870 and 1881 collected 343 cases of intestinal carcinoma in the Vienna General Hospital, of which 7 were in the duodenum and 10 in the ileum (none in the jejunum). From 1882 to 1893 he reported 243 cases of intestinal carcinoma, three cases of sarcoma and nine cases of lymphosarcoma. The sarcomata were of the round cell and spindle cell types and were located one each in the ileum, cecum, and rectum. The lymphosarcomata were located, one in the duodenum, three in the jejunum, three in the ileum and two in the cecum. Pick cited several cases of primary lymphosarcoma of the duodenum, and Trevers reported a case of primary melanosarcoma of the ileum. Hecktoen (1897) reported a case of simple hemangioma in the upper part of the small intestine. Rolleston (1901) re-

^{*} Received for publication, December 8, 1938.

ported an annular stenosing carcinoma of the third portion of the duodenum. Sutton (1901) stated that between the pylorus and the ileocecal valve, carcinoma is rare, and when present the duodenum is the most common site. Bunting (1904) records a case of multiple primary carcinoma of the ileum and cites three similar cases from the literature. The tumors were chance findings at autopsy, small sessile polypoid growths projecting into the lumen with the overlying mucosa movable, and none showed any metastases. Histologically they are characterized by a slow growth of undifferentiated cells with infrequent mitotic figures and a well developed fibrous stroma.

Lahev (1915) reported two cases of annular adenocarcinoma, one in the jejunum and one in the ileum and cites 4 similar cases in the ileum, two reported by Lubarsch and two by Müller. Soper (1929) in a roentgenographic study of lesions of the small intestine cites a case of an annular carcinoma of the jejunum with mesenteric lymph node metastases in a woman aged 60. and a case of spindle cell sarcoma of the jejunum with mesenteric lymph node metastases in a woman aged 43, who recovered after having a recurrent tumor of the ovary resected a year later. Rankin and Mayo (1930) collected from the Mayo Clinic over a ten vear period 31 cases of carcinoma of the small intestine. During that time there were 2775 cases in the large intestine and rectum and 2646 cases in the stomach. Up to 1919 there were 55 cases of carcinoma of the small intestine, 4597 cases in the large intestine and rectum, and 4335 in the stomach. Thirtyseven of these were in males, eighteen in females. The age group of these tumors varied between 32 and 69 with an average of 47.5 years. Ewing's average is 46.5 years. Nickerson and Williams (1937) reviewed the literature and reported ten cases of primary malignant tumors of the small intestine found at autopsy in the Mallory Institute of Pathology, Boston City Hospital during the past 40 years. Of this series two were cases of myosarcoma of the duodenum and eight were cases of carcinoma, six in the duodenum and two in the jejunum. The carcinomata, with the exception of one in the jejunum, all showed metastases. The sarcomata did not show any metastases. Five

TABLE 1 RESUMÉ OF NEOPLASMS OF THE SMALL INTESTINE

STATUS OF PATIENT	Died after chol- ecystduo- denostomy	Died after intes- tinal anasto- mosis	Died after intes- tinal anasto- mosis	Alive 2 years after resection	Died from car- diac incompe- tency	Alive 74 years after resection	Survived re- moval of perito- neal recurrence 34 years later	Died following resection	Died from carcinoma of oesophagus	Alive 14 years after resection
MICROSCOPIC DIAGNOSIS	Slowly growing cylindri- cal cell type adenocar- cinoma	Slowly growing gelatinous papillary adenocarcinoma, cylindrical cell type	Slowly growing gelatinous adenocarcinoma	Slowly growing adenocar- cinoma cylindrical cell type	Slowly growing carcinoid or argentaffin tumor	Smooth muscle fibers in- terspersed by cells with processes and giant cells. Leiomyosarcoma.	Slowly growing spindle cells with processes arranged in whorls. Leio-myosarcoma	Large cells with processes and giant cells. Many mitotic figures. Leio- myosarcoma	Small and larger hyalin- ized vessels separated by smooth muscle fibers in palisade arrangement.	Dilated lymph vessels lined by endothelial cells and lipoid giant cells. Microcystic lymphan-
METASTABES	Regional lymph- nodes	None	None	None	Mesenteric nodes and liver	None	Mesentery and omentum	None	None	None
GROSS DESCRIPTION	Oval crateriform ulcer 3 x 2 cm. around ampulla of Vater extending into pancreatic duct	Villous, papillary growth around the ampulla of Vater, 5 cm. in diameter	Nodular mass 3 cm. in dia. in the terminal portion of the duodenum with adhesions producing an "hour-glass" stomach	Stenosing tumor 1 cm. dia. with dilatation prox- imally	Small indurated ulcer 15 mm, in diameter 1 mm. deep	An extraluminar oval tu- mor 11 cm. long x 9 cm. dia. composed of fleshy and cystic areas	Nodular mass 12 cm. in diameter with extensive ulceration	Sessile polypoid growth 3 cm. dia. producing intustusception of the jeinnum	Nodular growth 2 cm. in diameter	Multiple small cysts scat- tered over entire ileum
SITE OF TUMOR	Second portion of duodenum	Second portion of duodenum	Third portion of duodenum	Jejunum	Jejunum	Jejunum	Jejunum	Jejunum	Jejunum	Tleum.
CLINICAL FEATURES	Jaundice, asthenia, nausea, loss of weight	Anemia, periodic attacks of jaundice, anorexia, abdominal distress	Nausea, belching, indiges- tion, vomiting, loss of weight, anemia	Abdominal cramps, nausea Jejunum and vomiting six months duration	Chance finding at autopsy. No symptoms referable to this lesion	Abdominal mass three months. An acute ab- domen two days	Painless abdominal mass 15 years	Acute intestinal obstruc-	None. Found at explora- tory laparotomy	Constipation, abdominal cramps, tympany
SEX	[iz ₄	(See	íu,	14	M	14	14	M	E4	M
AGE	63	62	72	49	42	20	8	22	8	33
NO.	-	69	69	*	19	60		00	•	10

surgical cases of malignant neoplasm were encountered by them during the same period of time but not included in the study.

after resection

tered over entire ileum

This report is based on a study of ten cases of primary neoplasms of the small intestines encountered during the past 8 years in the Newark Presbyterian Hospital. There were 23,426 surgical cases with 194 neoplasms of the gastrointestinal tract. The incidence of neoplasm of the small intestine is 5.1 per cent which is slightly higher than the figure in the literature. These figures include only those cases from whom material was sent to the laboratory for diagnosis, or was found at autopsy.

In this series, three neoplasms were in the duodenum, six in the jejunum and one in the ileum. Examination of the tumors shows four adenocarcinomata, three leiomyosarcomata, one hemangiomyoma, one lymphangioma, one argentaffin tumor.

The three adenocarcinomata of the duodenum include one in the third portion near the ligament of Treitz and two in the second portion, one accompanied by jaundice, the other free from jaundice. The six in the jejunum include three leiomyosarcomata, one annular stenosing adenocarcinoma, one hemangiomyoma and one argentaffin tumor. The tumor in the ileum was a lymphangioma multiforme microcysticum.

DISCUSSION

The first case is that of a female 63 who had a typical ulcerated adenocarcinoma around the ampulla of Vater, with areas of colloid degeneration. The tumor produced obstruction to the outflow of bile, progressive painless jaundice and dilatation of the gall bladder, hepatic and pancreatic ducts. Death resulted from chronic cholangitis and suppurative pancreatitis. While the roentgenological report was "possibly a retrogastric tumor," the roentgenograms showed a peculiar pattern closely resembling the ulcerative lesion in the duodenum (fig. 6 and 7). This is worthy of notice since it is only by observing the pattern produced in a number of cases that we may arrive at a definite diagnosis.

The second case is that of a female 62 who had a gelatinous papillary adenocarcinoma around the ampulla of Vater with only partial obstruction to the outflow of bile. For several years the patient had occasional attacks of jaundice, vague gastric symptoms and pernicious anemia was suspected. It is interesting to note that the obstruction which could not be relieved by intestinal anastomosis was caused not by the neoplasm but by a retroperitoneal hernia of two loops of jejuno-ileum. Roentgenograms showed a lesion in the duodenum with proximal dilatation but no obstruction (fig. 4).

The third case is that of a female 72 who had a fibrosing adenocarcinoma of the horizontal or third portion of the duodenum near the ligament of Treitz producing intestinal obstruction with dilatation of the duodenum proximally. The roentgenogram showed obstruction in the terminal end of the duodenum and an "hour-glass" dilatation of the stomach. The latter was produced by a fibrous band rather than by an intragastric lesion as is usually the case. This is an unusual site for duodenal carcinoma since most of the cases recorded occurred in the first and second portions.

The fourth case is that of a female 49 who had a small annular stenosing adenocarcinoma of the jejunum, which produced obstruction and dilatation of the proximal portion of the intestine (fig. 2). The tumor was successfully resected, with post operative recovery, and the patient is alive and well two years later.

These four cases of carcinoma of the small intestines ranged in age between 49 and 72 years. They were all in females which is rather unusual, since, statistically they are more common in males. The site of these tumors is also unusual as they are supposed to be more numerous toward the pylorus above, and the ileo-cecal valve below. They are all slowly growing tumors of the cylindrical cell type. The first three showing areas of colloid degeneration and regional lymph node metastases while the fourth case showed no visible metastases. In none of these four cases was there any hepatic invasion. This type of carcinoma should be easily diagnosed roentgenologically, and early resection should be successful.

The fifth case is that of a carcinoid or argentaffin tumor of the jejunum in a male 42 years of age who died of cardiac incompetency and the tumor was found at autopsy. While the patient showed no symptoms referable to the small ulcerated lesion in the jejunum, there were metastases in the regional mesenteric lymph nodes and in the liver. These tumors are very seldom found in the small intestine and only very rarely do they metastasize. The cases of Lubarsch, Bunting and Oberndorfer are, according to Ewing, benign embryonal multiple carcinoids of the small intestine. Aschoff describes benign and malignant carcinoids. Karsner states that the cells in these tumors are epithelial in character but are agentaffin cells as shown by Masson, derived from the periglandular nerve plexus. Klemperer reported ten cases of argentaffin tumors, some of which are in the small intestines.

The sixth case is that of a leiomyosarcoma of the jejunum in a female 59 years of age who was aware of an abdominal mass for three months and came for relief only when an acute abdomen was produced by rupture of a hemorrhagic cyst of the tumor (fig. 5). In spite of this rupture and of numerous adhesions, it was successfully resected and the patient is alive and well seven years later.

The seventh case is that of a leiomyosarcoma of the jejunum in a female 66 years of age who was aware of an abdominal mass for 15 years. In spite of extensive ulceration, mesenteric and omental metastases resection was successful, and the patient is alive three and a half years later, when she was again operated upon for peritoneal recurrence of a cystic leiomyosarcoma, and returned home relieved of her symptoms (figs. 8, 9, and 10).*

These two cases again emphasize that myosarcoma is a relatively benign tumor and early or even late surgical removal is apt to be successful. In this connection it is well to bring out the fact stressed by several authors that repeated examinations of the stool for occult blood are very useful in arriving at a diagnosis of neoplasm of the small intestine.

The eighth case is that of a peculiar polypoid sarcoma of the jejunum in a male 72 years of age who developed acute intestinal obstruction from intussusception and died following the resection. It was a firm tumor and the cells were irregular in size, had proc-

^{*} Nine months later a similar recurrence was successfully resected.

esses, and numerous mitotic figures. The nuclei were oval and polymorphous. It is probably a leiomyosarcoma (figs. 11 and 12). This tumor was seen by several eminent oncologists and those who ventured an opinion agree with this diagnosis.

The ninth case is that of a benign tumor of the jejunum in a female 60 years of age, and because it was composed of definite simple and cystic vascular areas as well as areas of smooth muscle fibers, it is designated as hemangiomyoma (figs. 1 and 3). The patient also had at the same time a carcinoma of the lower oesophagus to which she later succumbed. Moore and Schmeisser in 1934 reported three cases of benign tumors of the small intestine. After reviewing the literature they concluded that fibroma and myoma are more common than hemangioma.

The tenth case is that of a multiple cystic condition of the ileum in a male 33 years of age who had vague gastrointestinal symptoms for a number of years during which time an appendectomy, and later a cholecystectomy were performed without relief. This condition is probably congenital. The histological picture is that of a telangicatatic or microcystic lymphangioma of the submucosa containing numerous lipoid giant cells (figs. 13 to 16).

Intestinal emphysema in swine resembles closely the condition found in this case. In the animals the cysts are much larger, pedunculated, mostly at the mesenteric border involving all of the coats of the intestine, the mesentery, and mesenteric lymph nodes. Cysts lined by giant cells are rare. Biester, Eveleth, and Yamashiro (1936) found that intestinal emphysema in swine is due to a deficiency, and produced this condition by a diet containing a considerable amount of polished rice. This condition was produced only in those pigs that gained weight rapidly.

This patient had a varied diet with a considerable amount of milk and only rarely a little rice. The material from this case was seen by seven eminent oncologists with the following opinions: Three favored the diagnosis of lymphangioma, two thought it to be a congenital anomaly of the lymph vessels, and two thought that it is a condition similar to "intestinal emphysema" in swine. In general, it fits Ewing's description of a lymphangioma, and is best described by the term of lymphangioma multiforme microcysticum.

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Kaufman (1929) states that adenomyomas are occasionally found in the duodenum and jejunum, polypoid myoma is rarely seen, and lymphangioma is very rare. Aschoff (1923) describes a lymphangioma as occurring in the form of whitish spots in the mucosa of the small intestine. According to Bell (1938) all forms of lymphangioma are usually congenital.

The prominent symptoms in the cases of carcinoma were: nausea, anemia, weakness, loss of weight, and various signs of obstruction. One case not included in this series, that of a stenosing adenocarcinoma of the jejunum in a woman 50 years of age, diagnosed Roentgenologically and found at laparotomy to have extensive hepatic metastases, showed only nausea, anemia, and weakness. Two of the myosarcomata showed no other symptoms except an abdominal mass, indicating that clinically also they are relatively benign.

SUMMARY

- 1. Ten cases of neoplasm of the small intestine are recorded: seven in females and three in males.
- 2. Of the four carcinomata two were in the second portion of the duodenum, one in the third portion of the duodenum and one in the jejunum.
- 3. Of the six other neoplasms, three were leiomyosarcomata of the jejunum; one, a hemangiomyoma of the jejunum, one a metastasizing argentaffin tumor of the jejunum, and one a multiple microcystic lymphangioma of the ileum.
- 4. Two of the cases of jejunal leiomyosarcoma were successfully resected, one even in the presence of metastases.

Since this report was submitted for publication, two cases of lymphosarcoma were admitted. One case was that of a rapidly growing lymphosarcoma in a male 15 years of age who had symptoms of abdominal distress and nausea for about one year. A piece of intestine, 58 cm. long, including the ileum, cecum and ascending colon was resected. There was a bulky growth on the ileum side of the ileo-cecal valve producing intussusception into the cecum and colon. Microscopic examination showed small round cells with numerous mitotic figures invading the wall of the terminal ileum and ileo-cecal valve, and extending along the subserosa to the edges of the resected colon and ileum. The patient died 30 days after the operation.

The other was a case of reticulum cell lymphosarcoma of the jejunum in a female 71 years of age who had symptoms of abdominal distress, cramps and tympany for about six months. A piece of intestine 34 cm. long was resected, showing an annular growth 4 cm. long projecting into the lumen 3 cm., producing complete obstruction. The adjacent lymph nodes were enlarged. Microscopic examination showed reticulum cells with numerous mitotic figures invading the wall of the intestine. The patient recovered and was discharged 36 days after operation.

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DESCRIPTION OF PLATES

Fig. 1. Longitudinal section of angiomyoma of jejunum. Case 9. Note the mucous folds that are flattened but not involved in the growth. Two-thirds natural size.

Fig. 2. Longitudinal section of annular stenosing adenocarcinoma of jejunum. Case 4. Note sharp line of demarcation between the tumor and the jejunal wall. Natural size.

Fig. 3. Hemangiomyoma of jejunum. Case 9. Note the smooth muscle fibers between the blood vessels. \times 100.

Fig. 4. Roentgenogram of a duodenal gelatinous papillary adenocarcinoma. Case 2. Note filling defect in dilated second portion of the duodenum.

Fig. 5. Leiomyosarcoma of the jejunum. Case 6. Note undifferentiated cells separating smooth muscle bundles. \times 144.

Fig. 6. Roentgenogram of a periampular ulcerating adenocarcinoma, frontal view. Case 1. Note peculiar defect distal to the duodenal cap.

Fig. 7. Roentgenogram of a periampular ulcerating adenocarcinoma. Lateral view. Case 1. Note semilunar defect distal to the duodenal cap.

Fig. 8. Longitudinal section of cystic leiomyosarcoma of jejunum. Case 7. Note ulceration, large extraluminar cystic mass, and omental nodules. One-half natural size.

Fig. 9. Leiomyosarcoma of jejunum. Case 7. Note whorls of irregular fibers with elongated nuclei. \times 250.

Fig. 10. Leiomyosarcoma of jejunum. Case 7. Note irregular size and shape of fibers. \times 1000.

Fig. 11. Leiomyosarcoma of jejunum. Case 8. Note line of demarcation between the growth and normal submucous and muscle coats. The mucosa over the tumor is intact and the mucosal folds appear flattened. × 12.

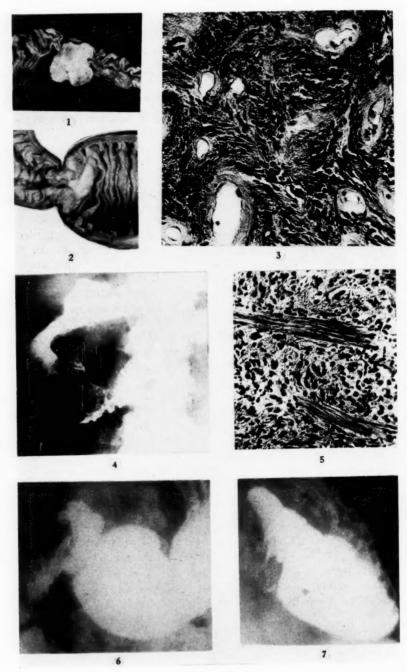
Fig. 12. Leiomyosarcoma of jejunum. Case 8. Note irregularity of size and shape of the fibers, and atypical large nucleoli in places. \times 1000.

Fig. 13. Lymphangioma of ileum. Case 10. Note groups of cysts scattered over the greater curvature of the dilated ileum. Three-quarters natural size.

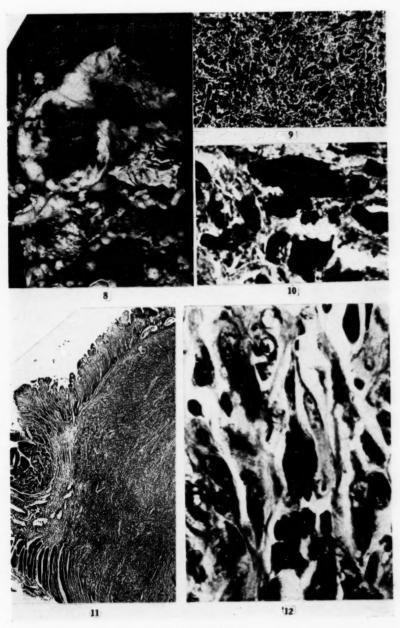
Fig. 14. Lymphangioma of ileum. Case 10. Note buds of lymph vessels, surrounded by endothelial cells and lymphocytes. \times 250.

Fig. 15. Lymphangioma of ileum. Case 10. Note cystic appearance of dilated lymph vessels, some of which are lined by flattened endothelial cells, while others are surrounded by lipoid giant cells. × 100.

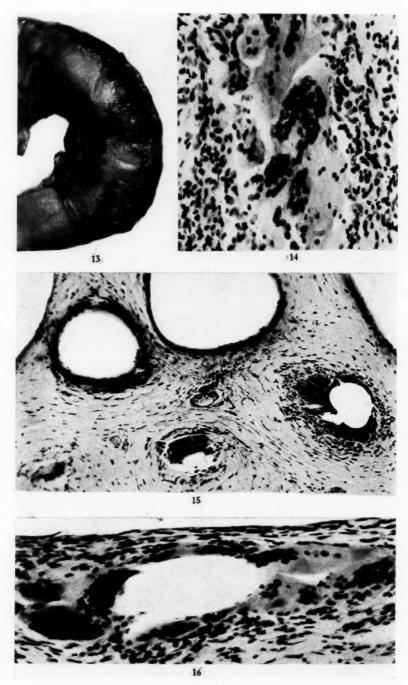
Fig. 16. Lymphangioma of ileum. Case 10. Note cystic appearance of a dilated lymph vessel lined by peculiar endothelial giant cells. \times 250.



Figs. 1-7



Figs. 8-12



Figs. 13-16

EDITORIAL

A CALL TO BROADEN LABORATORY MEDICINE

It is trite to state that we should broaden our usefulness but it is always appropriate to consider how it may be done, especially when a new call is made upon us. Events of recent years have made it clear that poisonings both acute and chronic, both industrial and in routine life or medical episodes, from both organic and inorganic substances, have become more and more the subject of great concern to the internist in the refinements of differential diagnosis. All kinds of toxic substances are meant of which a few will illustrate the point—arsenic, mercury, opium. It is no news to readers that the establishment of the diagnosis of chronic lead poisoning is fraught with the greatest difficulty and that opium derivatives may have almost mystic effects. The rising interest in industrial diseases has brought to the fore the fact that internal medicine, with its indispensable ally, laboratory medicine, will be called upon with increasing frequency. Johnstone in the Journal of the American Medical Association (111:1741) emphasizes that the whole medical fraternity must participate in the solution of the problem, which is not a surgical one alone as it has been so long considered. The part that can be played by the laboratory is in the appreciation by its officers of the possibilities in chemical, serological and cytological analyses that bear upon the effect of toxic substances. Of course many workers examine for mercury in the urine, make the estimation of sulphanilamide in the blood or trace the course of stippling in the red cells. But as a section of the work, clinical toxicology is poorly driven. This is due to many things. Requests are few; clinicians often fail to think of the field or suppose that the laboratory is ill equipped or not interested. The lack of interest may indeed be born of paucity of requests but is is surely supported by a realization that tests are difficult to perform because of questionable methods and expense of apparatus. Infrequency of usage makes for uncertain results since it is undoubtedly true that occasionally performed tests are less dependable than those

refined by long practice, those we are set up for and which run smoothly. To counteract these reasons greater familiarity is to be encouraged in order to perfect and simplify methods or to devise new ones. This Journal is helping very greatly in this direction by the maintenance of a technical section, to which Friend has recently made a notable contribution of his standard methods that are directly employed in the Peter Bent Brigham Hospital.

While the foregoing might represent the development within the rooms of the laboratory for toxicological tests, the preparation of the individual officers as doctors of medicine and as citizens may play a part in our usefulness in the greater field. While it cannot be gainsaid that satisfactory methods are indispensable, the relationships with other factors are to be evaluated. Association with the clinician is likewise indispensable since not every intoxication with the same thing will have the same effect. Much depends upon clinical care. It has been shown for example that more lead is stored in the body when calcium intake is low than when it is high. Certain inorganic substances lead to tissue insensitivity and reduced excretion. In the use of measures to detect poisons, especially where methods and interpretations depend upon so many factors, preliminary consultation and preparatory steps by clinician and laboratory officer become imperative. Collaboration of this kind with the internist need not obscure the calling, indeed we know that it will not dull the charm, of laboratory medicine. It may indeed help to develop that general field of toxicology so little nurtured in our country. Too much of such work seems to be left to commercial laboratories, and worthy many of them are, but the human application is lost. Or it is driven by industrial plants, medical circles being deprived of interesting and instructive contacts. Much work of a social or political nature is turned over to governmental agencies, particularly in this time of social 'insecurity', again to the detriment of those who practice, privately or in hospitals, their chosen branch. Finally, it is certainly not improper to wish for the broadening of our abilities and facilities for experience and proper private gain.

HERBERT FOX

OBITUARIES

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Dr. Carl Boettinger was born in 1879. He received his medical degree from Cornell University Medical College in 1903.

He was Clinical Professor of Medicine at New York University College of Medicine, a member of the City Board of Health, Visiting Physician to St. John's Long Island (N. Y.) Hospital and the Mary Immaculate Hospital, Jamaica, N. Y., and Visiting Physician and Director of the Medical Service, Queen's General Hospital.

Dr. Boettinger was a member of the House of Delegates of the American Medical Association in 1935–1936, a Fellow of the American College of Physicians, a member of the Society of American Bacteriologists and past president of the Medical Society of Queen's County.

He died on February 1, 1939 at his home in Forest Hills, N. Y. of coronary thrombosis, at the age of fifty-nine.

Dr. Samuel Thompson Lindsay was born in 1884. He graduated from Harvard University Medical School in 1923. He served on the Staff of the St. Mary's Hospital of Rochester, New York. He was a diplomate of the American Board of Pathology.

Dr. Lindsay was a man of unusually strong character, with a brilliant and logical mind and excellent judgment. He was at all times ready to aid and instruct the younger members of the profession.

He died of appendicitis on October 10, 1938, in Rochester, New York, at the age of fifty-four.

DR. WILLIAM HENRY SEEMANN was born in 1878 and obtained his premedical education at Immaculate Conception College, New Orleans. He graduated from the Tulane University of Louisiana School of Medicine in 1900 and later studied at the University of Liverpool.

Long prominent in both the teaching and practicing fields of medicine, he was professor of Hygiene and Public Health at Tulane University School of Medicine and previously professor of Hygiene and Tropical Medicine at New Orleans Polyclinic. For the past twenty years he had been pathologist for the city and state boards of health. At one time he was also city bacteriologist. He was also pathologist for the New Orleans Eye, Ear, Nose and Throat Hospital.

He was a member of the American Medical Association House of Delegates in 1915–1916, 1919–1926, 1930–1938. At the 1938 session of the American Medical Association in San Francisco he accepted the first Distinguished Service Medal of the Association in behalf of Dr. Rudolph Matas, New Orleans, who was chosen to receive the award but was unable to be present at the meeting.

Dr. Seemann was a past president of the New Orleans Parish Medical Society and of the Louisiana State Medical Society.

He died on November 19, 1938, at the age of sixty.

NEWS AND NOTICES

CONVENTION NOTES

From all reports the 1939 convention was the finest yet held. All those who attended were outspoken in their praise of the local arrangements and agreed that the program was outstanding in interest and excellence. The attendance was in keeping with the excellence of the meeting. Registrations numbered 175 and does not include all who were present. The Seminar was attended by 119 and 185 were present at the banquet. Both scientific and commercial exhibits, of which there were 31, were of commendable character and attracted well-deserved attention.

The Ward Burdick Award was presented to Dr. Harry Goldblatt for his work on Hypertension.

Drs. S. E. Ziffron, C. A. Owen, G. R. Hoffman and H. P. Smith of Iowa City, Iowa were awarded the Gold Medal for Excellence in Scientific Exhibit for their work on A New Simplified Prothrombin Test.

The Scroll for Meritorious Service was awarded to Dr. Philip Hillkowtiz.

The following were elected to membership:

Albano, Edwin, East Orange, New Jersey.

Allen, Hollis N., St. Louis, Missouri.

Benner, Miriam C., Denver, Colorado.

Choisser, Roger M., Washington, D. C.

Collier, William D., Youngstown, Ohio.

Connerty, Harold V., Washington, D. C.

DeMonbreun, William A., Nashville, Tennessee

Grauer, Robert C., Pittsburgh, Pennsylvania.

Grave, Floyd, Minneapolis, Minnesota.

Gray, Samuel H., St. Louis, Missouri.

Oray, Samuel II., St. Louis, Wissouti

Jetter, Walter W., Buffalo, New York.

Kriz, Joseph R., New Orleans, Louisiana.

Larson, Charles P., Fort Steilacoom, Washington.

Margolin, Ellis S., Sykesville, Maryland.

McGary, Lester, Madison, Wisconsin.

McCurdy, Gordon A., Victoria, B. C., Canada.

Morris, Joyce S., Binghamton, New York.

Neale, Richard C., Richmond, Virginia.

Norton, Louisa M., Concord, New Hampshire.

Rose, S. Brandt, Philadelphia, Pennsylvania.

Ross, Elizabeth, Pittsburgh, Pennsylvania.

Shanks, George, Toronto, Ontario, Canada.

Sunderman, F. William, Philadelphia, Pennsylvania.

Tomlinson, Wray J., Clinton, Iowa.

Turcotte, Hector, Lauzon, Quebec, Canada,

Warner, E. D., Iowa City, Iowa,

Weintraub, Solomon, New York, New York.

Zeman, E. D., Belleville, Illinois.

Associate Membership:

Tolstoi, George, Washington, D. C.

The newly elected officers and members of Committees follow:

President-Elect: Dr. A. V. St. George. Vice-President: Dr. Charles L. Klenk.

Secretary-Treasurer: Dr. A. S. Giordano (term: 3 years).

Executive Committee: Dr. T. B. Magath, Dr. F. W. Konzelman (term: 3 years).

Board of Censors: Dr. F. B. Queen, Dr. W. R. Mathews (term: 3 years).

Board of Registry: Dr. A. H. Braden, Dr. J. B. McNaught (term: 3 years). Dr. R. A. Kilduffe was re-appointed as Editor of The Journal for a term of 3 years and Dr. W. S. Thomas was re-appointed as Editor of The Technical Supplement.

Attractive in format, and informative as well as interesting in content, The Laboratory, the little monthly bulletin issued by the Fisher Scientific Co., Pittsburgh, Pa., is of definite interest to the laboratory worker. It will be sent on request.

In the Medicine and Public Health Building, New York World's Fair, Lederle Laboratories are sponsoring the scientific exhibits on Allergy and on Pneumonia, each exhibit being controlled by a committee of eminent specialists on these diseases. All exhibits in the building are scientific in character, merely carrying on a small plaque the names of the sponsors.

The Pneumonia exhibit, surfaced entirely of white laminated "Beetle," occupies a booth 20 x 30 feet in a commanding position. It presents, pictorially, the best composite opinion of the medical profession on how a pneumonia case should be treated. The narrative is unfolded by means of a sequence of dioramas, pictures, and charts. The story begins with an 'animation' of a man walking in the rain, and takes him through typing and serum therapy and all the various progressive stages of a typical case of pneumonia to a final picture at the serum farm where his little daughter is pictured, saying, "Thanks, old horse, you saved my Daddy's life!" A "Postscript" deals with Sulfapyridine.

The second exhibit, on Allergy, tells, in changing dramatic sequences, three 2 minute dramas of Allergy: "Tommy Todd's Autumn 'Colds'," "Mrs. Tucker's

Wheezes" and "Baby Bing's Eczema."

By means of an animated question box and dioramas showing typical

scenes in the doctor's office, a search for the offending allergic excitant in each of the three stories is conducted through information obtained by questions, scratch tests and an examination of the patient's family tree. An interesting part of the allergy exhibit is an illuminated transparency chart showing in full color, 48 of the most common allergic excitants. A separate series of little pictures invites the visitor to examine commonplace scenes for causes of allergy and then, by pressing buttons, to illuminate the concealed answers.

Both exhibits are addressed to intelligent laity, and are attracting close attention.

Physicians visiting the New York World's Fair are entitled to exclusive privileges in the Professional Club in the same building. Admission is obtained by simple identification as a doctor, without charge, and is only available to physicians and their guests. Provision is made here for consultation with exhibit sponsors on technical questions.

Bulletin and Announcement of Division of Cancer Control, Department of Health, Commonwealth of Pennsylvania, John J. Shaw, M.D., Secretary of Health

Cancer is now a reportable disease in Pennsylvania, but this in itself will not make possible the accumulation of sufficient data on which to base a rational program of progress in cancer control for the citizens of the State. For such a purpose it is necessary that many more facts be known and correlated.

There are many questions of immediate practical importance which can be answered if only the proper data can be collected and correlated. Such important problems as the value of preoperative and postoperative irradiation in cancer of the breast, the value of irradiation of the lymph nodes of the neck when they are not clinically the seat of metastases from cancer of the lower lip, the general distribution of cancer by age and sex, and the extreme importance of delay time, all can be solved by the accumulation of proper data.

Recognition of these facts has been given by the Medical Society of the State of Pennsylvania. In the annual report of its cancer Commission for 1938, the general principles were laid down showing how a fact-finding program could be organized for such a program, and the provisions were ratified by the House of Delegates of the Society.

To this end, then, it is proposed to augment the simple reporting of cancer cases by collecting data for every tumor case in the State of Pennsylvania. The word tumor is here defined as any neoplastic growth, benign or malignant, to include Hodgkin's disease, the lymphomas, leukemias, proliferative cysts (this includes retention cysts) teratomas, etc. In addition, a microscopic slide preparation from all cases in which biopsy has been performed or specimens removed, and in some a block of tissue of the cancer, are to be sent to the State Department of Health Laboratory, 34th and Locust Streets, Philadelphia. Appropriate containers will be provided for the mailing of these specimens and the data.

The specimens and data will be assembled and classified in the State De-

partment of Health for periodic review and the information derived will be distributed through the Medical Society of the State of Pennsylvania and its Cancer Commission for recommendations as to better organization and correlation of cancer treatment facilities in the State. A final check will be made with the death certificates as they are collected in the Bureau of Vital Statistics.

In all cases in which an autopsy is performed, microscopic sections of the

growth and data are to be sent to the same place.

Pathologists occupy the key position in this accumulation of data because the best means of diagnosis of cancer is by pathological examination. To cover the cost of preparation of extra slides and of filling out blanks, the sum of fifty cents will be paid for each slide plus blank received.

This will not be a diagnostic service, nor will the diagnoses of individual pathologists be criticized or superseded. In cases of differences of opinion, these will be recorded on the cards in the files for final disposal on the basis of further data, or as a result of conferences. These conferences, to be attended by pathologists and others interested, will be held at stated times, tentatively set at once a year, preferably at the time of the meeting of the Pennsylvania State Medical Society.

All individual cases, rare or commonplace, remain under the jurisdiction of the physicians in attendance on the patient, and will not be used except as part

of mass statistics.

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Obviously, the names of patients are essential for proper disposal and classification of data. Assurance is given to all clinicians and patients that names will be held inviolate and will be used only for the necessary cataloging and by only such few persons as are necessary for this purpose, who will be honor-bound to hold inviolate the names of patients.

Such a fact-finding procedure will:

Make for keeping better records.
 Answer numerous immediately important practical questions, such as radiation before and after breast cancer surgery, delay-times, surgery vs. radiation in lip cancer, etc., etc.

3. Stimulate the taking of biopsies.

- 4. Make for better pathological diagnoses.
- 5. Raise all standards of diagnoses and treatment.

6. Educate doctors and lay people.

7. Provide data to determine the part heredity plays in the incidence of cancer.

This collection of slides and data will be available to any qualified person for study and correlation, at the discretion of the Secretary of Health. We urge your whole-hearted cooperation in filling out the official cards because the success of this investigation depends entirely upon complete answers to every question in the questionnaire.

The magnitude and importance of this work have resulted in the establishment, by the Secretary of Health, of a Division of Cancer Control in the De-

partment of Health of the Commonwealth of Pennsylvania.

BOOK REVIEWS

Life's Beginning On The Earth. By R. Beutner, M.D., Ph.D., Professor of Pharmacology at The Hahnemann Medical College and Hospital of Philadelphia. Cloth, 22 pp., 80 figures, \$3.00. The Williams & Wilkins Co., Baltimore, Md.

Few problems have been more fascinating to the human mind throughout the ages than the origin of life or have given rise to more varied concepts. Not only the scientists, but the philosopher ("cogito ergo sum") and the poet ("I came like Water, And like Wind I go") have wrestled with it; but whether life came into the world as an instantaneously created phenomenon or represents the product of a slow, laborious evolution through the eons is still a mystery to which the final answer as yet remains unknown.

In this book Professor Beutner presents a scholarly and fascinating discussion of the problem in its varied aspects and advances his own theory, one of great interest.

He believes that the appearance of life on the earth was simply a cosmic event resulting from the interplay of cosmic forces, or, in other words, that "life is just one of the countless properties of the compounds of carbon."

In support of this contention he discusses the present scientific understanding of life's phenomena, its working mechanisms and illustrates in a fascinating way the manner in which many so-called "vital forces" may be imitated and artificially produced.

His book is divided into four main sections: The First Approach: Vital Growth and Crystallization; The Second Approach: Life, Carbon's Outstanding Property; The Third Approach: The Importance of Salt and Water for Life and Growth; The Fourth Approach: The Animal A Machine.

Despite the complexity of the subject, the style is clear and simple and understandable by the average reader unfamiliar with chemistry or even with science in general to any degree.

This is a stimulating book of intriguing interest sure to awaken discussion and worthy of a place in any comprehensive library.

A Textbook of Medicine. Edited by Russell L. Cecil, A.B., M.D., Sc.D., Professor of Clinical Medicine, Cornell University Medical College, Associate Editor for Diseases of The Nervous System, and Foster Kennedy, M.D., R.R.S.E., Professor of Clinical Neurology, Cornell University Medical College. Cloth, Ed. 4, Revised and entirely reset. 1614 pp., 37 figures. W. B. Saunders Co., Phila., Pa.

The preceding editions of this book have so well established it as an outstanding text that it requires no further introduction.

The present edition, so extensively revised as to have been entirely reset, not only shows many changes throughout but includes many new subjects. With this edition, as a consequence of the establishment of a retiring age for contributors, many old subjects have been rewritten or revised by new authors, thus assuring "new blood" and a fresh outlook.

As before, this book can be recommended as an excellent and up-to-date text amply reflecting the present status of medicine. It is well organized, well written, comprehensive and authoritative and contains a good index making its contents readily accessible.

A Guide To Human Parasitology for Medical Practitioners. By D. R. BLACK-LOCK, M.D., D.P.H., D.T.M., Professor of Tropical Hygiene, Liverpool School of Tropical Medicine, The University of Liverpool, and T. SOUTHWELL, D.Sc., Ph.D., F.R.S.E., Lecturer in Parasitology, School of Tropical Medicine, The University of Liverpool. Cloth, Ed. 3, 259 pp., 122 figures, 2 colored plates, \$4.00. William Wood and Co., Baltimore, Md.

This may be described as a concise "refresher" text intended, primarily, for those whose contact with parasitic diseases is casual and occasional and those endeavoring to qualify for diplomas in Tropical Hygiene, Tropical Medicine

and Public Health.

As such, it presents in a clear, concise manner the salient characters of the common pathogenic parasites of man and the methods utilized for their detection and identification.

The authors have, therefore, laid emphasis on the pathogenic organisms and have largely restricted their descriptions to characteristics of diagnostic significance. The book contains many useful tables and an excellent set of diagrams illustrating in a clear and graphic manner the life histories of the more important parasites.

There are a few faulty type alignments here and there and an occasional

typographical error (e.g., Dibothriocephalus Slatus, p. 199).

This can be recommended as a useful book, not only for the physician and student, but for the laboratory worker as well.

Syphilis. Edited by Forest Ray Moulton. Cloth, 193 pp., 33 figures. The Science Press.

This volume contains the third symposium presented by the Section on Medical Sciences of The American Association for the Advancement of Science, the preceding symposia being upon *The Cancer Problem* (1937) and *Tuberculosis and Leprosy* (1938).

The present volume, containing thirty papers by thirty-two authors, is of fascinating interest and presents a clear, well documented summary of the

present knowledge of syphilis. As such, it is not only of exceeding interest, but of great value.

In a discussion of *The Holy Wood and The Haitian Myth of The Origin of Syphilis* R. C. Holcomb presents the evidence opposing the American origin of syphilis, while the next paper—*The Case for The American Origin of Syphilis* by W. A. Pusey—presents the evidence favoring this belief.

C. S. Butler upholds the case for the identity of syphilis and yaws; Howard Fox presents the opposing evidence and E. H. Hudson discusses Bejel.

The Spirocheta pallida is discussed from varied angles in three contributions by N. R. Ingraham, Jr., Thomas B. Turner and R. E. Olsen; and experimental syphilis is covered by Louise Pearce.

Space does not permit listing the other contributions in seriatim but it may be added that treatment, immunity, pathology and serological aspects are thoroughly covered.

All in all, this is a book of outstanding interest and value; one which may be read with pleasure and interest for its literary value and with profit for its comprehensive and authoritative summary of the present status of knowledge of this disease.

This is a volume of absorbing interest and exceeding value to the physician, pathologist, serologist, public health worker and, indeed, to all interested in syphilis—as, indeed, in these days, who is not?

This is one book the purchase of which is practically obligatory.

Scarlet Fever. By George F. Dick, M.D., D.Sc., Professor of Medicine, University of Chicago, Attending Physician, Billings Memorial Hospital, Editor, Department of Infectious Diseases, The Year Book of General Medicine; and Gladys Henry Dick, M.D., D.Sc. Cloth, 149 pp., 8 colored plates. The Year Book Publishers, Inc., Chicago, Ill.

This is a well organized and well written book comprising a comprehensive and authoritative discussion of scarlet fever.

The fact that it comes from the pen of the Drs. Dick who discovered the Streptococcus scarlatinae is first hand evidence of the authoritative character of the book. The text is clear, the style easy and the colored plates excellent.

The physician, pathologist, public health worker and all who are concerned with this disease will find this excellent book a valuable reference text.

Avian Tuberculosis Infections. By WILLIAM H. FELDMAN, D.V., M.M.S., Associate in Division of Experimental Medicine, Institute of Experimental Medicine; Associate Professor of Comparative Anatomy, Mayo Foundation for Medical Education and Research, Graduate School, University of Minnesota. Cloth, 483 pp., 109 illustrations, \$7.00. The Williams & Wilkins Co., Baltimore, Md.

This is, perhaps the most comprehensive monograph yet published concerning avian tuberculosis infections, and as such should be of great interest and value to all who, directly or indirectly, are concerned with this subject.

Avian tuberculosis is a subject of interest and importance, not only from the economic standpoint but from the scientific aspect as well because of its similarity in many respects to bovine and human tuberculosis.

In this volume Professor Feldman has covered the subject in an admirably comprehensive manner; not only the literature, which is critically digested; but also the practical, clinical, laboratory and pathological features of the disease. The book bears the hallmark of an ample experience and will be the standard reference text on this subject for years to come. It can be highly recommended as a thorough, complete and authoritative study.

Synopsis of Laboratory Methods. By W. E. Bray, B.A., M.D., Professor of Clinical Pathology, University of Virginia; Director of Clinical Laboratories, University of Virginia Hospital. Cloth, Ed. 2, 408 pp., 51 text illustrations and 17 color plates, \$4.50. The C. V. Mosby Co., St. Louis, Mo.

This is an excellent book. Compact, concise without being laconic, it covers the field of laboratory procedures in relation to disease in a very thorough

and comprehensive manner.

Dr. Bray's experience as a teacher is amply reflected in his book for his descriptions are clear and understandable and evidence thorough and long familiarity with his subject.

Not only the medical student and student technician, but the clinical pathologist and physician will find this little volume a good working companion and a ready reference text as well. The illustrations are exceptionally good; well chosen and excellently reproduced.

This new edition reflects the advances since the first and includes a variety

of new procedures.

Bray's manual may be highly recommended as a multum in parvo deserving of a cordial reception and wide circulation; altogether an excellent book.

Cancer, With Special Reference To Cancer Of The Breast. By R. J. Behan, M.D. Dr.Med. (Berlin) F.A.C.S., Co-founder and formerly Director of The Cancer Department of The Pittsburgh Skin and Cancer Foundation, Pittsburgh, Penn. Cloth, 844 pp. 168 illustrations, \$10. C. V. Mosby Co., St. Louis, Mo.

This book is primarily addressed to the clinician seeking to enlarge his knowledge of the cancer problem and is based upon a comprehensive survey of the literature concerned with the etiology, diagnosis and treatment of cancer.

While Dr. Behan has reviewed the advances which seemed to be based upon substantial foundations, he has not neglected to mention others which have not—perhaps, as yet—received general acceptance. And, in a book of this kind this is as it should be for this is not presented as other than a comprehensive survey of the subject designed to stimulate an interest in the problem.

While there may be those who will disagree with Dr. Behan on one thing or another—a happening all medical authors must expect—none can read his

book without becoming a bit the wiser in one way or another. A medical book which achieves that has achieved its purpose.

Cancer—Its Diagnosis and Treatment. By Max Cutler, M.D., Associate in Surgery, Northwestern University, Medical School; Chairman, Scientific Committee, Chicago Tumor Institute; Consultant, Tumor Clinic and Director, Cancer Research, United States Veterans Administration, Hines, Illinois; and Franz Buschke, M.D., Assistant Roentgenologist, Chicago Tumor Institute; Late Assistant, Roentgen Institute, University of Zurich. Assisted by Simeon T. Cantril, M.D., Director, Tumor Institute, Swedish Hospital, Seattle; Late Assistant, Chicago Tumor Institute. 757 pp., 346 illustrations, Cloth, \$10. W. B. Saunders Company, Philadelphia.

As stated in the Preface, the purpose of this book is "to present the essential clinical features of the more common forms of cancer" and, particularly, "to make accessible to the reader a critical evaluation of the pertinent facts in the diagnosis, prognosis and treatment of cancer as gleaned from the world literature and reviewed in the light of our own experience."

That this purpose has been amply fulfilled the book bears witness. Realizing the importance of diagnosis, the authors lay particular emphasis on this phase of the problem.

This volume may be highly recommended as an excellent, thorough, comprehensive and well planned survey of a most important problem. Pathologist, physician, and specialist alike should have this work as a reference text.

Your Chest Should Be Flat. By S. A. Weisman, M.D., F.A.C.P., Assistant Professor of Medicine, University of Minnesota; Member Consulting Staff, Glen Lake Sanatarium, Oak Terrace, Minnesota. Cloth, 145 pp., 74 illustrations in 49 figures, \$2. J. B. Lippincott Co., Philadelphia.

This is a very thoughtful and constructive little book based upon an extensive series (20,000) of chest measurements and devoted to the proposition that the deep chest makes a better soil for tuberculosis. It will undoubtedly arouse interest in the practical use of the thoracic index.

Dr. Weisman's evidence controverting the old axiom that the typical tubercular chest was flat and sunken in in comparison with the round, deep chest of health, is not only extensive but well organized and digested.

This is a thoughtful, well planned study and a constructive contribution to the story of tuberculosis and the book should be of interest and value to all who are interested in tuberculosis: physician, nurse and even the layman.

Human Pathology. By Howard T. Karsner, M.D., Professor of Pathology,
Western Reserve University, Cleveland, Ohio. With an introduction by
SIMON FLEXNER, M.D. Ed. 5, Cloth, 1013 pp., 461 illustrations, 18 in color.
J. B. Lippincott & Co., Philadelphia, Pa.

Those familiar with this book will not be surprised to find it entering upon

a fifth edition. Those who are not will not be long in appreciating its excellence.

Although not increased in size this new edition bears evidence of thorough revision and many changes as indicated, in part, by the inclusion of nearly 250 new references. Among the changes are discussions of the significance of pulmonary silicosis, the newer views on edema, thrombosis, pericardial adhesion, circulatory disturbances and cardiovascular disease. The text upon the hematopoietic system has been largely rewritten as has also that upon diseases of the pancreas, tuberculosis, pulmonary diseases and diseases of the central nervous system. Six new plates have been added and many illustrations replaced.

As before, Karsner's Pathology may be accepted as a well written, thorough and comprehensive standard text of value to student, practitioner and pathologist alike.

Laboratory Manual of Hematologic Technic. By Regena Cook Beck, M.A., M.D., Formerly Instructor in Pathology and Bacteriology at George Washington University Medical School; Head of the Department of Bacteriology, William and Mary College Extension; Pathologist to Stuart Circle Hospital and Director of the Stuart Circle Hospital School of Medical Technology, Richmond, Va. With a Foreword by Frank W. Konzelman, M.D., Professor of Clinical Pathology, Temple University, Philadelphia. 389 pp. 79 illustrations, Cloth, \$4.00. W. B. Saunders Co., Philadelphia.

This is a very excellent and practical manual intended primarily for the training of the laboratory technician now better known as the medical tech-

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Dr. Beck is too well known as a hematologist and clinical pathologist of repute to require introduction. Her book bears ample evidence both of her experience and her ability as a teacher.

While intended for use as a teaching manual, this book may well be used by the medical student, interne and physician and as a useful reference text in the laboratory.

It can be recommended without reserve as covering the field of hematology in an excellent, clear and thorough manner.

CORRESPONDENCE

May 1, 1939

The Editor
American Journal of Clinical Pathology
Mount Royal and Guilford Aves.
Baltimore, Maryland

Dear Sir:

Under the Federal Food, Drug, and Cosmetic Act the standards of strength, quality and purity laid down in the Pharmacopoeia for the drugs and preparations that it recognizes become the legal standards for such drugs and preparations. As a consequence the manufacturer, the dispensing pharmacist and the physician have a common interest in the Pharmacopoeia. The manufacturer is enabled to furnish the pharmacist with officially standardized materials, the pharmacist to dispense, with exactitude, just what the physician desires, and the physician to write his prescriptions in simple terms with confidence in what the pharmacist will dispense. Without the Pharmacopoeia there would be chaos. Without confidence in its sponsors the situation would be perilous.

The Convention for the Revision of the Pharmacopoeia decides the principles under which the Pharmacopoeia is to undergo revision. It also elects the officers of the Convention, a Board of Trustees to manage administrative, legal and financial matters, and a Committee of Revision, all to serve until the next Revision Convention meets.

The Committee of Revision is composed of fifty elected members. Seventeen of these are doctors of medicine, representatives of clinical medicine, pharmacology, serology, therapeutics, etc. The other thirty-three members belong to Pharmacy and the allied sciences, and include representatives of dispensing and manufacturing pharmacy, inorganic and organic chemistry, botany, pharmacognosy, biological assay, etc.

In the past the Committee of Revision has included men of the highest rank in the several fields. That it may continue so to do, it is asked that the various bodies authorized to send delegates to the Convention will appoint their full quota of delegates, and will select these from among those of their own people whom they known to be informed and at the same time prepared to attend the Convention.

Cordially yours,

Walter A. Bastedo, M.D.

President of the U. S. P. Convention
1930-1940

May 1, 1939

In compliance with the provisions of the Constitution and By-Laws of the United States Pharmacopœial Convention, I hereby invite the several bodies entitled under the Constitution to representation therein to appoint three delegates and three alternates to the Convention for the Revision of the Pharmacopœia of the United States of America, which is to meet in Washington, D. C., on May 14, 1940.

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Walter A. Bastedo, M.D., President of the United States Pharmacopæial Convention. ARTON A MARK

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PREPARATION OF UNIVERSALLY COMPATIBLE ASCITIC FLUID FOR TRANSFUSION*

ROGER M. CHOISSER AND ELIZABETH M. RAMSEY

From the Department of Pathology, School of Medicine, The George Washington University, Washington, D. C.

Previous studies with human ascitic¹ fluid having demonstrated its efficacy in restoring and maintaining the blood pressure level in dogs subjected to experimentally induced shock, further work has been carried out in the endeavor to extend the field of usefulness of this readily available therapeutic agent.

As indicated by the results of the preliminary studies, the protein content of ascitic fluid and its bacteriostatic properties make it a peculiarly suitable substance for use in combatting lowered blood volume of whatever etiology. The presence of type specific red cell agglutinins in high titre, however, necessitated typing of the recipient's blood and the ascitic fluid to be used prior to transfusion. The series of experiments herewith reported was designed to discover, if possible, some way of obviating the necessity for this time consuming procedure.

The classic method of globulin precipitation with ammonium sulphate was studied but discarded as impractical in the present instance, due to the high dilution of the fluid consequent to dialysis. Attempts, as previously reported, were made to destroy the agglutinins by heating to 65°C. for protracted periods of time, but the results were variable and by no means dependable. Next, supersaturation of the fluid with sodium chloride was carried out but was also found to be an impractical method as albumen was precipitated as well as globulin, unless preliminary quantitative analysis of albumen-globulin ratio of each specimen was made to determine the exact amount of sodium chloride required in each instance. Finally, plain dialysis against tap water was

^{*} Received for publication July 5, 1939.

tried and found to be effective in precipitating the globulins alone. Some 24 hours' dialysis was required to reduce 100 cc. of the fluid to the isoelectric point of globulin. If, however, an electrodialysis was set up using non-moisture-proof cellophane sacs for the fluid and brass or carbon electrodes connected with ordinary 110 volt Direct Current, 500 cc. of the fluid could be freed of electrolytes in 2–3 hours. The time required varies in accordance with the quantity dialyzed and the protein content of the given fluid. The precipitate of globulin so obtained is exceedingly finely divided. In the majority of instances it can be completely removed by passage through a double thickness of \$2\$ Whatman filter paper. Occasionally it can only be eliminated by Berkefeld filtration.

Removal of salts in the process of dialysis brings about an increase in the pH of the fluid as a result of which small floccular "shreds" are frequently formed when the fluid is tested against human cells of any type. If the pH of the fluid be restored to 7.5 with normal HCl, this phenomenon is abolished and it is found that the electrodialysis and filtration have entirely removed the agglutinins present in the original sample. The increase in volume of the sample is minimal, in no instance so far studied in excess of 10 cc., so that this method is free of the criticism that it excessively reduces the protein content of the fluid. Furthermore, the Berkefeld filtration insures the sterility of the fluid, which it is our custom to neutralize before filtration, and subsequently to transfer directly from the sterile filtration flask to sterile ampoules in which it is sealed and stored in an ice box until required for use. That the agglutinins are entirely eliminated and not merely suppressed has been established by tests performed after 6 months of storage.

The accompanying chart shows the results of agglutination tests and protein and hydrogen ion concentration determinations before and after processing 500 cc. samples of 18 different specimens of ascitic fluid.

It has been found that occasional specimens of ascitic fluid which contain no agglutinins for human cells do, however, contain some subgroup which agglutinates dog cells, hence we deem it advisable to electrodialyze all fluid to be administered to experimental animals. It is our routine to pool a convenient number of specimens, regardless of type, filter to remove mucus and cells and determine the protein content of a sample of the mixture. If the value is below 2.5 gm./100 cc. we concentrate it

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CHART 1

SPECIMEN NUMBER	UNTREATED			ELECTRODIA- LYZED 2 HOURS			NEUTRAL- IZED		ELECTRODIA- LYZED 3 HOURS			NEUTRAL- IZED			
	Agglu- tinins	Protein gm. %	Hd	Hd	Agglutinins	"Shreds"	Ηd	Agglutinins	"Shreds"	рН	Agglutinins	"Shreds"	Hq	Agglutinins	"Shreds"
21	A B			8.65+	_	+	7.5	_	_						
22	AB	1.09	7.9	8.65 +	-	+	7.5		-						
24 Lot 1*	0	1.22													
24 Lot 2	0	1.58	7.9												
24 Lot 3	0	1.12		8.65 +	-	?	7.7	-	_						
25	0	0.90	8.5	8.5		-									
26 Lot 1	A		7.3	8.65 +	-	+	7.5	-	-						
26 Lot 2	A	0.70	7.6	8.3	-	+	7.5	-	_						
26 Lot 3	A	0.61	7.7	8.65 +	_	+	7.7	_	-						
27 500 cc.	A B	1.04		8.65+	-	-									
27 750 cc.	AB	1.04			+	+				8.65+	-	+	7.5	-	-
28	A B														
29	A B g	0.87			_	_									
30	A BA														
31 Lot 1	AB	0.78			_	_									
31 Lot 2	AB	3.13			+	+				8.65+	_	+	7.5	_	-
32	В	1.28			+	+				8.65+	_		7.5	_	-
33	В	2.75			+	+				8.65+	_		7.5	-	

^{*} Lot numbers refer to successive paracenteses performed upon the same patient.

prior to electrodialysis by an adaptation of the pervaporation method of Terry².

Eight dogs in which shock had been induced by hemorrhage were treated with ascitic fluid, processed as described above. In all eight cases the red cells of the animal's blood were promptly and strongly agglutinated by untreated samples of the specimen of ascitic fluid used for infusion, regardless of type. Yet in none of the cases did the processed fluid, administered intravenously, cause immediate or remote reactions of any sort. The restorative effect of the fluid as evidenced by rise in blood pressure and maintenance of the increased level was of the same order as that observed in the preliminary experiments with ascitic fluid previously reported.

SUMMARY

Ascitic fluid may be freed of agglutinins and rendered safe for intravenous use in relief of experimental shock in dogs, without the necessity of preliminary cross matching with the animal's blood. This is accomplished by a process of electrodialysis, adjustment of the pH and subsequent Berkefeld filtration in accordance with the method described above.

REFERENCES

- Choisser, R. M., and Ramsey, E. M.: Use of ascitic fluid in the treatment of primary shock. Proc. Soc. Exper. Biol. and Med., 38: 651-652, 1938.
- (2) Terry, M. C.: Artificial concentration of test serums in blood grouping. J. Am. Med. Assoc., 112: 135-136, 1939.

SILENT, SO-CALLED PRIMARY TUBERCULOSIS OF THE SPLEEN*

HERBERT FOX

From The Pepper Laboratory of Clinical Medicine, University of Pennsylvania

The following is a record, together with a list of references most useful in study of the cases of three cases of silent, so-called primary tuberculosis of the spleen, diagnosed by splenectomy, in which the tubercle bacilli could not be demonstrated by acceptable laboratory methods. The clinical material was from the services of Dr. Pepper, Dr. Eliason, and Dr. Ferguson, at the University of Pennsylvania Hospital.

Male, 51. Loss of 20 per cent of weight in 18 months. General health otherwise good. Duration of splenomegaly not known. No history of tuberculosis, personal or familial. Blood count within normal limits. Reported in good health three years after operation.

Female, 21. Loss of weight during two years prior to an injury during the examination for which splenomegaly found. History of nervous imbalance and hypochondria, none of tuberculosis. Clinical thought of splenic anemia. Reported in good condition two years after operation.

Female, 38. Failing health with no particular symptoms for 1½ years. Splenomegaly first observed two months before operation. Slight enlargement of liver. No indication of tuberculosis, historically or clinically. Splenic anemia considered from anemia, thrombocytopenia and lymphocytosis. Constant improvement since splenectomy.

A description of these spleens follows with which is combined the picture to be found in the literature.

The organ varies in weight from 300 to 3300 grams, but this weight is not in relationship to the known history of duration or to the extent of the histological lesions. The color is deep red-brown or brown-purple. The capsule is

^{*} Received for publication February 16, 1939.

smooth and rarely shows evidence of subacute capsulitis. The subcapsular tissue is delicately mottled without distinct graphic scheme. The cut surface is moderately bloody, with distinct but not increased trabeculations and clear but not increased vascularity. Scattered through the pulp are poorly outlined areas like irregular, poorly defined follicles that prove to be miliary tubercles. True follicles are relatively reduced, grossly and minutely. It seems that the follicular centers are the places of origin of the tubercles. Under the microscope one finds epithelioid masses with imperfect giant cells and but little lymphocytic bordering, or well defined milia enclosing numbers of Langhan's giant cells. Fibrous tissue is sometimes plentiful, as a ring about the tubercles, or increased diffusely throughout the organ. Caseation was not complete in our cases and its absence is often emphasized in the literature.

Numerous bacterial stains, and the guinea pig method for case 2, failed to reveal the presence of tubercle bacilli. Tuberculosis was not suspected because of the history and the appearance of the organ. These facts were also true in the cases of Engelbreth-Holm, Hickling, Gloor, and others. Cynman emphasizes the difficulty of demonstrating organisms. Many cases have however been proven as tuberculosis.

Tuberculosis of the spleen has been divided into the acute and chronic forms; with the acute, purpura, pernicious anemia, hemolytic icterus and leukemia have been associated; with the chronic forms, polycythemia and the Banti's syndrome have been associated. Pathologically, tuberculous splenitis has been divided into the gross caseous form, most often associated with easily demonstrable lesions elsewhere, and the miliary form with fibrosis, not associated with demonstrable tuberculosis elsewhere. This latter is the form often called primary and for which the present name of "silent" is offered. Cynman speaks of isolated, independent, miliary tuberculosis of the spleen.

The most important clinical features of this chronic, miliary and fibrous splenomegaly are the absence of clinically demonstrable tuberculosis in the body, the association with blood dyscrasias under the guise of polycythemia and Banti's syndrome, and the unreliability of the tuberculin test. This splenic condition seems never to have been diagnosed before operation.

Histologically, the tuberculous granulation tissue and milia are

entirely acceptable. On three occasions milia have also been found in the liver at operation. Only one case coming to autopsy seems to be entirely free of some minor focus but an advancing lesion is not recorded. Winternitz records one case that failed entirely to show lesions in other organs.

Hereto are attached the most important references that were used in solving the cases and an abstract from the article of Fittipaldi which is believed to represent European, especially the Italian, viewpoint to students in this country, in a language not usually available.

FITTIPALDI, C. Considerazioni anatomiche sulla tubercolosi della milza con particolare rignardo alle splenomegalie tubercolosi. (Anatomic study of tuberculosis of the spleen with special reference to tuberculous splenomegaly.) Hamatol. Arch., 19: 75–109, 1938.

Primary tuberculous splenomegaly is extremely rare. Since the first reports by Colly in 1846 and Monneret in 1857, only about 80 cases have been reported including those from the Italian literature by Palumbo, Grillo, Ciaccio, Soth, Bulfalini, Marini, Sarakani, Villa, Luisada, Greppi e Reitano, Fabris, Sala. Not all admit the existence of a primary tuberculosis of the spleen in the true anatomo-pathologic sense, and some writers (Bloch and Letten) deny the possibility of a primary invasion of the spleen by the tubercle bacillus, stating that in cases with splenectomy (Cynman, Leider, Grillo, Bufalini, Sala, Bouffart, Martin) other tuberculous foci could not be excluded because of absence of clinical symptoms and that careful pathologic study would no doubt have revealed foci in other organs. Autopsies in such cases have in fact revealed tuberculous foci in the lungs, lymph glands and intestines (Askanazy, Lubarsch, Pol, Esser, Leidel). Such foci might be old or in a state of very low activity.

Greppi insists that the involvement of the spleen is so predominant that splenic tuberculosis must be considered as one of the clinical, primary splenopathies.

The present writer agrees with Greppi not only because the clinical symptoms are limited almost exclusively to the spleen, but because the blood picture and the hemorrhagic manifestations disappear following splenectomy. In the blood picture the erythrocytes show the predominant changes, the leukocyte formula is inconstant with a certain degree of polynucleosis or lymphomononucleosis. The total number of leukocytes is usually normal or slightly increased. In a case reported by Reitano, splenic tuberculosis was associated with acute lymphatic leukemia.

Many cases of tuberculous splenomegaly are associated with polycythemia which represents one symptom of the Rosengart triad (splenomegaly, poly-

cythemia and cyanosis) described as characteristic of primary tuberculous splenomegaly by the French. Most cases have some degree of anemia of moderate hypochrome type (with scant signs of regeneration) which may become slowly progressive in chronic cases. In the acute febrile type of tuberculous splenomegaly, the anemia grows rapidly worse becoming hemolytic and toxic (Greppi). Rieux and Le Bourdelles suggest that the productive and destructive processes affecting the erythrocytes act in tuberculous splenomegaly so as to establish a sort of equilibrium. When destruction predominates there is marked anemia. When production predominates there is hyperglobulia. Besides acute terminal purpura, cases of chronic purpura have been reported.

The author agrees with Greppi that one cannot speak of a primary tuberculous splenomegaly in the strict anatomopathologic sense, but rather with Suenin and Baudet of localized splenic tuberculosis or better still with Roch of a tuberculosis predominantly of the spleen.

Cassano has shown that chronic tuberculin intoxication produces a marked mesenchymal reaction in the spleen with hyperplasia of the reticulum, whereas Achard and Weil et al. describe tuberculous splenomegaly as due to formation of granulomatous lesions.

As regards pathogenesis it is evident that the enlargement of the spleen is not due exclusively to formation of granuloma but also to non-specific lesions, such as thickness of the reticulum, congestion, hyperplasia of the cells of the pulp, accentuation of hemolytic phenomena, etc. associated in large measure with the tuberculous toxicosis, which may precede granuloma formation (pretubercular splenomegaly).

The lesions produced in tuberculous splenomegaly are classified as follows:

A. Localized or predominant: (1) Miliary: small nodules, large nodules;
(2) caseous: fibrocaseous, abscessed, pseudocystic. B. Secondary to more active tuberculous foci: (3) Necrotic-hemorrhagic, (4) fibrous or sclerotic, amyloid.

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EFFECT OF INGESTED SODIUM CHLORIDE ON CONCENTRATION OF HEMOGLOBIN*

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Diurnal variations of hemoglobin content have been reported by numerous investigators. Dreyer, Bazett and Pierce¹ have found that in apparently healthy individuals the hemoglobin varies considerably not only from day to day, but also during the same day. Rabinowitz² reports a drop of 26 per cent within two hours, with a return to the initial point within the following two hours. It is possible that an intake of an excessive amount of salt was the cause of a sudden drop in hemoglobin in some of the subjects.

The absorption by the blood of sodium chloride in hypertonic solution from the intestines is accompanied by a diffusion of water from the tissues into the blood and of the salt from the blood into the tissues. This exchange of salt and water between the blood and the tissues tends to increase temporarily the volume of blood with consequent dilution of blood. Iversen³ found that after giving a normal man 10 grams of sodium chloride in 200 cc. of water, the blood volume increased five per cent. Similar observations have been described by others.^{4, 5}

Since the increase of blood volume is associated with a decrease of blood solids, it is the aim of this study to observe to what extent the ingestion of sodium chloride affects the concentration of hemoglobin in the blood.

The study has been carried out on twenty healthy individuals, male and female, between the ages of eight and thirty-two years. From 5 to 15 grams of sodium chloride in 500 cc. of water were given one hour after a light breakfast.

^{*} Received for publication, March 23, 1939.

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TABLE 1
HEMOGLOBIN CONTENT OF BLOOD BEFORE AND AFTER INGESTION OF SODIUM CHLORIDE

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14.1
15.3
14.7
13.2
6.91
12.5
16.7
13.2
13.2
15.2
_
15.2
12.3
63
6

During the test, no brisk exercise was allowed. Additional water was allowed, as much as was desired. The hemoglobin was determined from capillary blood just before the ingestion of salt, then for six hours thereafter at hourly intervals, and also twenty-four hours after the ingestion of salt.

Hemoglobin determinations were made by the acid hematin method, using this author's colorimeter⁶ and a permanent glass standard previously checked against blood, the hemoglobin content of which was determined by Van Slykes' Oxygen Capacity method. A dark blue filter was used to facilitate matching of colors. Each determination was carried out in triplicate and the average taken. All determinations and colorimetric readings were done by the same person as follows:

To 6 cc. of N/10 hydrochloric acid, 20 cmm. of capillary blood was added by means of Sahli pipette. The mixture was shaken and kept in warm water (75°C.) for six minutes, to bring out the full color of acid hematin, then cooled under tap and examined in the colorimeter. The results are expressed in grams of hemoglobin per 100 cc. and also in percentage, 16.92 grams, being one hundred per cent—a figure found by Williamson⁷ as a normal average in a study of one thousand healthy individuals.

Generally, it would be better to discard the expression of hemoglobin in terms of percentage. The reason it was retained in this work is that it is easier to visualize variations of hemoglobin when expressed in percentage. Table 1 shows the hemoglobin content before ingestion of sodium chloride, the amount of salt ingested, hemoglobin concentration during the first 6 hours after ingestion of sodium chloride at hourly intervals, the amount of hemoglobin after twenty-four hours, and the percentage of maximum drops of hemoglobin from the initial reading.

COMMENT

- 1. In a study of twenty apparently healthy individuals, the ingestion of from 5 to 15 grams of sodium chloride in 500 cc. of water invariably was followed by a drop of hemoglobin content of the blood.
- 2. The average fall of hemoglobin from the initial reading was 11.2 per cent.
- 3. The maximum fall was 19 per cent in three subjects. The minimum fall was 2 per cent.
- 4. The ingestion of 5 and 6 grams of salt caused a smaller drop in hemoglobin in three out of four cases. The maximum drop of

hemoglobin (19 per cent in three individuals) was after the ingestion of 12, 15, and 10 grams of salt, respectively.

5. The maximum drop of hemoglobin was between three and four hours after ingestion of salt. After five hours, the hemo-

globin began to return to the initial point.

6. Since the ingestion of salt in hypertonic solution is followed by a fall in concentration of hemoglobin, it would be of interest to clinicians to ascertain whether the patient ingested appreciable amounts of salt with food, from three to six hours prior to determination of hemoglobin.

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LEIOMYOFIBROMATOSIS; MULTIPLE TUMORS OF ABDOMEN AND PELVIS*

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A survey of medical literature reveals numerous reported cases of extrauterine "fibroid tumors" which almost without exception are single tumors apparently originating in the connective tissue or muscle coats of the gastro-intestinal tract. Not a few arise from the uterine adnexa. They fall into two principal groups: fibromata^{1,2,5,6,9,10,12,13} and myomata.^{3,4,8,11} There are found a few reported cases of multiple neurofibromata, but these latter structures have no bearing on this case report.

The majority of myomata described produce some distortion of the associated viscera, a few project into the lumen of the intestine; a few are reported as pedunculated and project into the peritoneal cavity. There is a striking scarcity of case reports describing multiple smooth muscle tumors in the viscera outside the uterus and adnexa, although Ewing states that miliary myomas of the peritoneum may occur during the course of larger tumors. The following case is reported because of its rarity and unusual features.

CASE REPORT

Mrs. R. U., aged 35 years, white, married, no pregnancies, came to the hospital April 23, 1939 because of abundant and persistent uterine bleeding, weakness, and enlargement of lower abdomen. Her present complaint had its beginning several years ago with prolonged and copious menstruation and periods of intermenstrual bleeding which had become more abundant during the past six months. Her past history, aside from the usual childrens diseases, was entirely negative.

Physical examination showed a moderately well nourished woman showing no disturbances of the cardio-respiratory or gastro-intestinal systems. A mass

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filling the pelvis was felt on vaginal and rectal examinations. The neurological examination was entirely negative.

Laboratory examinations revealed nothing of significance: Hemoglobin 43 per cent (Sahli); erythrocytes 4.25 millions; leucocytes 9,100 with 82 per cent polynuclears. The blood film showed a microcytic, hypochromic anemia. The blood Kahn and Wassermann tests were negative.

A laparotomy was performed April 25th, 1939, resulting in an hysterectomy, right oophorectomy, left subtotal oophorectomy and an incidental appendectomy.

Gross examination of surgical tissue showed a large nodular uterus amputated above the cervix, measuring 15 by 20 cm. and weighing 910 grams. It consisted largely of intramuscular and subserous fibroids varying in size from 5 mm. to

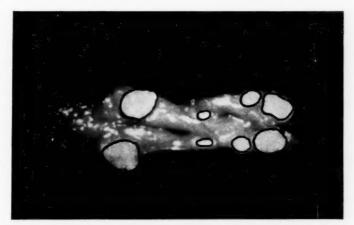


Fig. 1. Photograph of Appendix, Actual Size
The meso-appendix is split longitudinally bisecting four fibroid nodules.

5 cm. diameter, and of uniform density. There was one pedunculated submucous fibroid 3 by 5 cm. protruding into the endometrial cavity which was filled with clotted blood. The surface of this fibroid showed evidence of recent hemorrhage. Beneath the serosa were numerous minute fibroids 1 to 2 mm. each. The serosal surface was smooth over its entirety and there were no adhesions. There were several small encapsulated fibroid nodules in both broad ligaments and parametrial tissue averaging 1 to 3 mm. each. The appendix was 5 cm. long, itself negative to gross examination. A single small area of endometriosis with typical cytogenic stroma was found in the wall of the proximal end of the left Fallopian tube. In the meso-appendix there were four small encapsulated fibroid nodules 1 to 4 mm. diameter (see fig. 1). A similar fibroid mass from the peri-intestinal fat of the sigmoid was submitted to frozen section



Fig. 2. Section of a Small Nodule in the Meso-Appendix, Showing Bundles of Smooth Muscle, Separated by Connective Tissue Which Shows Progressive Hyalinization

for identification. Examination showed an encapsulated neoplasm composed of interlacing bundles of spindle shaped cells, whose arrangement and degree of differentiation were such that malignancy could be ruled out.

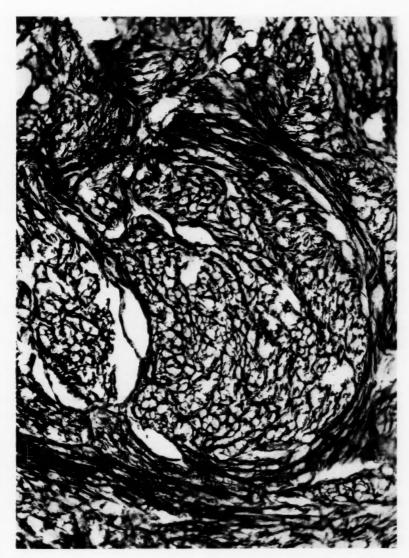


Fig. 3. Hortega Reticulum Stain (Silver, Gold Impregnation) Showing Transversely Cut Muscle Bundle Surrounded by Connective Tissue Fibrils and the Fibrillar Reticulum Surrounding and Supporting the Muscle Cells

This stain leaves the cut muscle cells unstained

Inspection of the abdomen and pelvis showed large numbers, literally hundreds, of the above described nodules which were widely distributed beneath and not upon the peritoneum of the pelvic floor, the broad ligaments, the omentum of the distal small intestine, the meso-appendix, in the peri-intestinal fat of the sigmoid and rectum. There were no adhesions, there was no evidence of invasion or formation of attachments. So far as could be determined from palpation (it was obviously impossible to examine all the lesions) the lesions had no attachment to the muscular coat of the intestine. This will be shown by Fig. 1 in which the nodules had no attachment to any of the coats proper of the appendix. It was obviously impossible to remove all the lesions because of their large number and distribution, and the abdomen was closed. Convalescence was entirely uneventful.

Histological examination: The numerous uterine fibroids were leiomyofibromata of the ordinary type, becoming progressively hyalinized as their size increased. There was no necrosis and no evidence of malignancy. Mitoses were not seen. The endometrium showed hyperplasia with a pattern of the mid-secretory phase. The endometrial stroma was congested and hemorrhagic. The tubes were histologically unimportant. The ovarian tissue showed a corpus luteum showing hemorrhage and involutional changes. The ovarian tissue otherwise was physiologic.

Sections taken through several of the small fibroid tumors showed typical mixed fibro-myomatous structure, similar to that seen in a typical uterine leiomyofibroma. In fact, were the source not known, one would be justified in calling them small uterine leiomyofibromata. They were characterized by whorls and bundles of smooth muscle, separated by whorls of connective tissue showing tendency to progressive hyalinization (see fig. 2). The smooth muscle showed the typical long rod shaped nuclei and it reacted with the yellow component of Van Gieson's stain. Hortega reticulum stain showed the muscle cells and bundles supported by a fine connective tissue reticulum (see fig. 3).

All the nodules examined showed an identical structure. No evidence of malignancy was seen. There was no necrosis. A diagnosis was made of multiple uterine and abdominal leiomyofibromatosis.

It is not within the scope of this report to theorize upon the origin of these many tumors confined to the lower abdomen and pelvis. That the lesions could be derived from the uterus seemed inconceivable, since there was no evidence of malignancy in either the uterine or the small abdominal masses. There was neither evidence of invasion beyond the margin of the masses nor invasion of the veins in either the uterine fibroids or the abdominal nodules. The lesions are obviously capable of a slowly

progressive growth, to what limits it is impossible to say. An attempt will be made to follow this patient's future course.

CONCLUSION

A case is reported of numerous abdominal and pelvic leiomyofibromata. Both gross and microscopic examination shows their benign character.

I wish to express my appreciation to Dr. Eslie Asbury, Cincinnati, Ohio for making this material available for study.

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SPINDLE CELL SARCOMA OF THE PROSTATE*

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Sarcoma of the prostate is reckoned as among the rarest of neoplasms. In the paper published in the British Journal of Urology in 1933 by Lowsley and Kimball of New York City, which contains the most recent exhaustive survey of literature, 132 cases—including the one reported therein—are listed. The earlier cases were mostly autopsy specimens, as diagnosis was not often made before death. These earlier cases are discussed in the article published by Charles A. Powers in 1908, wherein twenty-three cases (including his own), are listed. These were all "microscopically proven," although he adds ten which were "probably true cases," setting them aside from ten others regarded as "doubtful."

CASE REPORT

Mr. T. V., age 33, presented himself with the chief complaint of frequency, pain, and difficulty in urination. He had been well until about one year ago, when the difficulty in urination began to appear. Urine examination showed a trace of albumin, some casts, abundance of polymorphonuclear leukocytes and an occasional red blood cell.

Rectal examination revealed a large, irregular, soft mass. A prostatic resection was decided upon. There was considerable bleeding, and soft, mushy tissue was removed and sent to the laboratory for examination.

Pathological Report

Macroscopic examination: Pieces from the prostatic resection are many in number and weigh 22 grams. They are necrotic, vascular, exceedingly soft, and are of a dull gray appearance.

Microscopic examination: The histological characteristics of the prostate gland are entirely obliterated in these sections. There is a predominance of

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fusiform fibroblasts which are smaller than normal, very numerous, irregular in size, and show some mitoses. The nuclei are vesicular.

The Van Gieson stain substantiates the connective tissue origin of this neoplasm. No muscle cells were found.

Diagnosis: Spindle-cell sarcoma of the prostate gland.

The post operative treatment consisted of deep X-ray therapy. The patient continued to lose ground very rapidly with the development of cachexia, loss of weight, and pain radiating down the thighs, and died eight months after operation. Autopsy was not obtained.

The diagnosis of sarcoma of the prostate gland and its histological classifications have caused considerable disagreement among the pathologists.

Histologically, there are many different types of sarcoma of the prostate such as round-cell, spindle-cell sarcoma, rhabdomyosarcoma, myxosarcoma, and fibrosarcoma.

Ewing feels that the rhabdomyosarcoma is the only well defined variety and that the round and spindle cell sarcoma, the most frequent types, are also the least definite histologically.

The most frequent locations for metastases of sarcoma of the prostate are as follows: Bone, 11 per cent; lung, 11 per cent;

kidney, 8 per cent; liver, 8 per cent.

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In 1909 C. L. Gibson of New York reported to the American Association of Genito-Urinary Surgeons, that the number of "absolutely authenticated cases" was 37, including his own, that of Powers mentioned above, and some which had been published after Powers' list was compiled. The subject of Descums' thesis given at Toulouse in 1912 was sarcoma of the prostate, including a fairly complete review of literature, and another paper on the same subject, written by F. C. Herrick of Cleveland, brought the total of recorded cases up to sixty-two. In the fifteen years which intervened between the appearance of Herrick's paper, and the compilation of Lowsley and Kimball mentioned above, case reports of prostatic sarcoma—mostly solitary instances—became increasingly frequent. Faber's thesis, delivered at Jena in 1935 added another case, but contributed no new facts to the general subject, and some five or six others have appeared in literature during the past two years. But inasmuch as a grand total of less than 150 cases is on record the assertion that any form of prostatic sarcoma is very rare, still stands unchallenged.

In this comparatively small number of instances of prostatic sarcoma, the incidence of the spindle-cell type of growth is not high. In the table of Lowsley and Kimball, twenty-three cases, or 17.4 per cent, are listed as of this type. It is noticeable that Powell's case, published in 1928, is the last that is designated simply as "spindle-cell." The pathological reports made in the past ten years are, in general, more detailed in their examination of cell-structure and peculiar characteristics of neoplastic growths. Where earlier writers merely designated the results of microscopical examination by the name of the prevailing type of cell, at present we use the terms "leiomyosarcoma" or "rhabdomyosarcoma" to designate more precisely the pathological variations.

Etiology: As with most malignancies, the origin of prostatic sarcoma is as yet undetermined. But, as with most sarcomata. the incidence in the earlier years of life-in contrast to carcinomata which are rarely seen before middle age-gives confirmation to the theory of anomalous fetal development. Of the twenty-three cases of spindle-cell sarcoma mentioned above. three (13 per cent), were in infants under one year of age—those of Frankel, Laquire, and Bouchard, and Powell. Four (reported by Isambert, Wind, Bland-Sutton, and Putzu), were under ten years of age. Barth's patient was 17, Osgood's 19, Eastman's 27, Paschkis's first case 32, and Herrick's 33 years of The age of MacGowen's patient is not stated, so that only ten of the spindle-cell sarcomata were seen in individuals in that period of life commonly accepted as the "cancer age." This age of incidence corresponds quite closely with that for sarcoma seen in other portions of the genito-urinary tract, and thus serves to confirm the etiological theories held in regard to all such lesions.

Clinical manifestations: When seen early in life, sarcoma of the prostate, in common with sarcoma in other parts of the body, runs a rapidly fatal course. In older subjects, the progress is more gradual but sooner or later, the outcome is unfavorable. Some patients who underwent operation in middle life, have survived for a considerable period. The patient of Stern and Fein

lived for two years and that of Stein's, Ritter's, and Marsh's lived for two and a half years, while Eastman's patient, who was but twenty-seven when treated, survived for five years. In all the remainder the course was rapid, and the spread of the disease so extensive that death came as a release from an intolerable condition.

In most of the reports, the growth of the neoplasm is described as being upward and back underneath the vesical neck in such a way as to push the bladder forward and displace it to such an extent as to interfere with the outflow of urine both from the ureteral orifices into the bladder cavity and through the urethral outlet. The latter is the more common, the result being typical prostatic obstruction with its sequelae of retention, overflow incontinence, urinary infection, and serious or even fatal renal impairment.

The findings on rectal palpation are by no means characteristic, although the stony hardness associated with carcinoma of the prostate is not mentioned in most of the reports on physical examination. Some instances of confusion with prostatic abscess have been recorded, and in general, the consistency of the growth is soft and elastic, and in middle-aged or elderly subjects, may often be taken for benign adenoma. In young subjects, tuberculosis may be suggested, and it is always necessary to rule out a possible tuberculous condition before diagnosis can be established. In but few cases was a correct diagnosis reached before operation, although in a few of the more recently reported, the nature of the obstruction was recognized after modern urological diagnostic methods had been brought into service. Intravenous pyelography has proved helpful in several instances. Diagnosis, however, remains difficult and uncertain.

It is noted by Lowsley and Kimball in their discussion of all types of prostatic sarcoma that this particular growth is most frequently associated with an infiltration of the urinary bladder, seminal vesicles, and rectum, and less often of the external genitals and perineum. Regional extension they found to have occurred in more than 76 per cent of all the cases examined, most frequently in the round-cell type and next to that in the

spindle-cell type. The same was true of the occurrence of metastasis, the round-cell leading the spindle-cell in the frequency with which this was produced. But, "the spindle-cell sarcoma, apparently, is the most malignant; 44 per cent of the spindle-cell type form metastasises." These statements seem a little hard

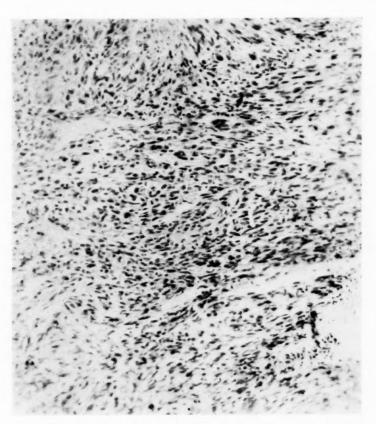


Fig. 1. Low-Power Photomicrograph Showing a Diffuse Growth of Fusiform Fibroblasts

to harmonize, but reference to table 1 (which is condensed from the larger ones presented by these authors), will show the correctness of the high incidence of metastasis when the spindle-cell type is considered by itself.

Prognosis and treatment: The prognosis in all types of prostatic sarcoma is uniformly unfavorable. What Gibson said a quarter

of a century ago—"This disease is fortunately rare, as so far it has defied all resource of surgical art to relieve it"—is still true today. The patient seldom comes under observation until the condition is well advanced, but even those few cases which have been seen early have not proved more amenable to treatment.

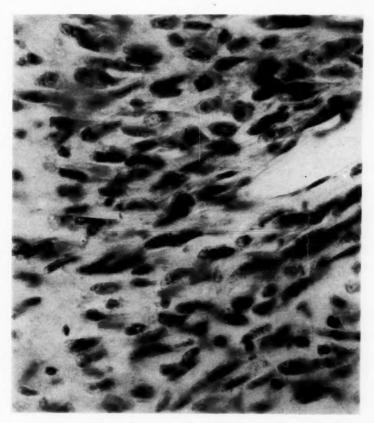


Fig. 2. High-Power Photomicrograph Showing the Fusiform Fibroblasts with Vesicular Nuclei and Some Mitosis

In middle-aged men, the complaint usually made has pointed to vesical neck obstruction, which is naturally taken for the prostatic enlargement frequently seen at that time of life. If competent urological consultants are at hand, differential diagnosis between benign or malignant enlargement will be quickly made, but even then the true nature of the obstruction may not be ascer-

TABLE 1
Cases of Spindle-Cell Sarcoma of the Prostate (Condensed from Lowsley and Kimball)

YEAR	AUTHOR	AGE	DURATION OF DISEASE	TYPE	METASTASES	
1853	Isambert	$8\frac{1}{2}$	7 months	Spindle-cell	Specimen only	
1888	Wind	$5\frac{1}{2}$	$3\frac{1}{2}$ months	Spindle-cell		
1883	West	21	1 month	Round and spindle-cell	(Not included in total spindle- cell cases)	
1891	Barth	17	9 weeks	Spindle-cell		
1896	Marsh	57	$2\frac{1}{2}$ years	Spindle-cell	To ilium	
1897	Bland-Sutton	7	Short time	Spindle-cell		
1898	McGowan	Not given	3 months	Spindle-cell		
1906	Frankel	10 months	5 months	Spindle-cell		
1908	Wolfgang and Biel	45	5 months	Spindle-cell	Lungs and retro- peritoneal nodes	
	Idem	46	3 months	Spindle-cell	Liver and kid- neys	
1909	Eastman	27	5 years*	Spindle-cell	No metastases but apparent extension to bladder. Some doubt as to origin in pros- tate	
1910	Paschkis	32	Brief	Spindle-cell		
1912	Pleschner	54	Brief	Spindle-cell		
1913	Osgood	19	2 months	Small spin- dle-cell		
1916	Schneider	49	18 months	Spindle-cell		
1917	Parmenter	69	7 months	Spindle-cell	Bowel and peri- toneum	
1920	Herrick	33	1 year	Spindle-cell	Liver	
1921	Stein and Ritter	45	$2\frac{1}{2}$ years	Spindle-cell	Abdominal wall	
1922	Putzu	8	6 months	Spindle-cell	Abdominal wall	
1923	Gruber and Maier	64	7 months	Spindle-cell	Kidney, ureter, and spleen	

N. B.: Seven cases of leiomyosarcoma presenting spindle-cell structure which appear in the original table are omitted here.

* Original article says "apparently running a course covering five years." There had been for five years increasing irritation of the urinary reflex . . . pain . . . etc.

TABLE 1-Concluded

YEAR	AUTHOR	AGE	DURATION OF DISEASE	TYPE	METASTASES
1924	Hermans Tashiro	71 60	Brief 4½ weeks	Spindle-cell Round and spindle-cell	Axillary and inguinal nodes. Spleen, liver, lungs, pleura, lymph nodes, etc. (not included in total)
1925	Stern and Fein	45	2 years	Spindle-cell	Abdominal wall
1926	Laquere and Bouchard	9 months	3 weeks	Spindle-cell	
1928	Powell	10 months	2½ months	Spindle-cell	

tained. In children and young men, abnormal enlargement of the prostate should at once suggest the possibility of sarcoma. It might be that early removal and suitable post-operative radiation might prove adequate to control the disease. The success which has attended such a course applied to sarcoma of other parts of the body would give ground for such a hope, but past results eloquently testify that the methods previously used were too late to be of any avail.

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EDITORIAL

THE HOSPITAL AND THE PATHOLOGIST

Regardless of size or bed capacity, the multiple functions of the modern hospital are indivisible, interlocking and closely linked with the general efficiency of the institution as a whole. They may be described concisely as: (a) The scientific and skilful care of the sick; (b) teaching as concerns the nurse, the interne, the staff and the community, and (c) research, as related to the evolution or evaluation of methods and the better understanding of the mechanism and manifestations of disease.

It is unnecessary to elaborate upon the close and essential—indeed, vital—relation of the pathologist and the clinical laboratory to the attainment of these objectives, for this has long been obvious; but it was only when plans for hospital standardization and approval became operative that the problem of furnishing adequate laboratory facilities came to the fore and engaged the attention of hospital administrators and pathologists alike.

The problem, in some respects, becomes a problem largely, among other factors, because of the inexorable law of supply and demand. For there are not—nor, indeed, has there ever been—an adequate supply of competent pathologists. As a result, many situations have arisen which for many and various reasons influenced by many and various factors, have been unsatisfactory to both the pathologist and the hospital.

All problems have varied aspects influenced by the particular or individual angle of view. While it may seem that the greatest problem of all is to bring divergent view-points to a more or less common focus experience has shown that this may well be best attempted—and often achieved—by friendly conference and amicable interchange of ideas.

That, in this particular problem, this has thus been largely

achieved is evidenced by the principles governing the relation between the hospital and the pathologist which have recently been promulgated by the American Hospital Association.

Advanced in a spirit of coöperation and the intent to solve the many problems inherent in the interrelationship in question, these principles are of basic significance and importance. They should be read and studied by every pathologist and, particularly, by every hospital pathologist.*

Covering as they do, all the phases which, at one time or another, have come under discussion, they furnish an adequate and satisfactory basis for the discussion of any particular or individual problem in the future.

That such problems will arise is inevitable. That they may well be settled on the basis of these principles there is little reason to doubt.

R. A. KILDUFFE.

^{*} See page 576.



JULIUS FRIEDRICH COHNHEIM, 1839-84

Julius Friedrich Cohnheim, 1839-84

When fifty-five years ago Cohnheim's brilliant and infinitely versatile labors were cut short at the early age of forty-five, men were still under the spell of his dynamic personality, feeling too close, perhaps, to the bitterness of death to attempt an evaluation of the changes which he had wrought, or a refutation of his more speculative doctrines. In the centennial year of his birth (July 20, 1839) the time is ripe to call to grateful remembrance Cohnheim's monumental contributions to medical science, which have become an integral part of present teaching, and to pay tribute to his ingenious and inspired discoveries which seemed then so revolutionary, yet to us to-day so long overdue. His signature is writ large across many pages of the story of pathology. Rediscovering the phenomena of diapedesis, he threw a flood of light upon the obscurities of the nature of inflammation. His production of tuberculosis in the transparent cornea of the rabbit astounded and convinced a skeptical world. His neat and valuable improvements in laboratory technique, such as staining muscle nerve endings with silver salts and corneal nerve endings with gold, and his method of freezing fresh preparations in microscopic work, have become universal property. After sixty-two years his "Lectures on General Pathology" remain refreshingly up-to-date.

A robust, energetic man with a fund of humor and irony, Cohnheim was a fluent, persuasive lecturer, who trained a host of distinguished pupils: Weigert, Lassar, Heidenhain, Neisser, Ehrlich, and Welch. A victim of gout, the last years of his life were clouded by its complications, and he died of chronic interstitial nephritis ("gouty kidney") on August 15, 1884.

WALTER R. BETT.

NEWS AND NOTICES

THE SOCIETY AND THE WAGNER BILL

At the recent convention the following resolution was passed:

RESOLVED that the American Society of Clinical Pathologists is opposed to the so-called Wagner Health Act introduced into the Senate of the United States on the ground that it is inimical to the best interests of the patients and the public health, and that it will operate to the end to lower the present high standards of medical care as now practiced in this country.

BE IT FURTHER RESOLVED that this resolution be spread on the minutes of the Society and published in the Journal of the Society and that a copy be sent to the American Medical Association.

PRINCIPALS OF RELATIONSHIP BETWEEN PATHOLOGISTS AND HOSPITALS

It is recognized that pathological and other laboratory services are essential elements in the diagnosis and treatment of disease and in its prevention and control; that a competent laboratory service under skilled direction is an essential element in the hospital; that the number of qualified pathologists is limited; and that many hospitals and communities are too small to maintain locally qualified specialists in this field.

In view of the current discussions concerning the relationships of pathologists to hospitals, and because of the desirability of protecting the public, of maintaining laboratory services of high efficiency and of safeguarding the interests of the hospitals, the following principles are hereby approved by the Board of Trustees of the American Hospital Association:

- The pathological and other laboratory services of the hospital are primarily for the benefit of the sick, for the advance of medical science and for the prevention and control of disease in the community.
- 2. Hospital services in pathology and other branches of laboratory work should be organized as a department, under the direction of one or more competent pathologists who should be responsible for all the laboratory services of the hospital. Such a chief, or chiefs, of the department should be a physician and, wherever possible, should be one who is a qualified specialist in pathology, preferably a diplomate of the American Board of Pathology or the equivalent body in Canada.
- 3. If, because of size or isolation of the hospital or for other reasons, a qualified pathologist is not available locally, some member of the general medical staff, trained in pathology or paying particular attention to the subject, should be appointed in immediate charge of the department. Under these conditions a consultation service should be

arranged for the department with a qualified pathologist or with another hospital or agency in which a qualified pathologist is in charge of the laboratory service.

 Recognition of the pathologist as a professional member of the medical staff of the hospital and as head of a hospital department is obvious.

5. The fact that certain laboratory services relating to diseases of public health interest are sometimes provided by laboratories of state, provincial or local health departments should not lessen the responsibility of the hospital to provide adequate laboratory service.

6. The preservation of the unity of the hospital and its component departments and activities is an essential administrative principle. This principle can be maintained in respect to the laboratory department without any infringement on professional rights or professional dignity.

- 7. The financial arrangements between a hospital and its pathologist should be such as will best meet the local situation, since no one type of relation is applicable or suitable in all instances. The basis of financial arrangement may be salary or commission or fees, or such other method, or combination of methods, as will meet most effectively the needs of the patients and community, of the individual hospital and of the pathologist.
- 8. Laboratory technicians (when not employed directly by the pathologist) should be on salary and should be employed by the hospital administration, although recommended by and responsible for their professional direction to the director of the department.
- 9. Hospitals and pathologists should recognize that their primary obligation is efficient service to the patient and the community, with the maximum economy that is consistent with quality of service. This principle should govern the arrangements between hospitals and pathologists and the financial arrangements with hospital patients, and the principles should be so applied that neither the hospital nor the pathologist should exploit the patient or each other.

The Annual Meeting of the New Jersey Society of Clinical Pathologists was held at Atlantic City, June 7, in conjunction with the Annual Meeting of the New Jersey State Medical Society.

The Executive Committee reported a close cooperation between the Society, the State Department of Health, and the State Medical Society in the formation of plans for the continuance of the pneumonia and venereal disease campaigns and in the plan for the inauguration of a cancer program in New Jersey.

The following officers were unanimously re-elected: President: Asher Yaguda, M.D., Newark, N. J.

Vice-President: Robert A. Kilduffe, M.D., Atlantic City, N. J.

Secretary: A. J. Casselman, M.D., Camden, N. J. Treasurer: A. R. Casilli, M.D., Elizabeth, N. J.

Chairman of Executive Committee: R. A. Kilduffe, M.D., Atlantic City, N. J.

BOOK REVIEWS

Immunity. Principles and Applications in Medicine and Public Health. By Hans Zinsser, M.D., John F. Enders, Ph.D., and LeRoy A. Fothergill, M.D., 5th Edition of "Resistance to Infectious Diseases." Cloths, 801 pp., \$3.50. The MacMillan Company, New York.

This book requires no introduction for "Zinsser on Immunity" has long been a familiar and classic text in the literature on this subject. Neither the medical nor the physician's library can be regarded as complete without this book for it is, perhaps, the clearest exposition of the subject extant. Recommended without reserve as an outstanding exposition of the practical application of the principles of immunity to clinical problems.

Atlas of Ophthalmic Ophthalmology. Prepared at the Army Medical Museum from Material in the Registry of Ophthalmology. By Elbert DeCoursey, Captain, Medical Corps, U. S. A., Pathologist to the Registry, and J. E. Ash, Lt. Col. Medical Corps, U. S. A. Curator. Leatherette, loose-leaf, Ed. 2, revised, 272 pp., numerous microphotographs, \$18.00 (\$15.00 to members; \$12.00 to libraries). The American Academy of Ophthalmology and Otolaryngology, Medical Arts Bldg., Omaha, Neb.

Atlas of Otolaryngologic Pathology. Prepared at the Army Medical Museum from Material in The Registry of Otolaryngic Pathology by J. E. Ash, Lt. Col. Medical Corps, U. S. A. Curator. Leatherette, loose-leaf, 2d Edition, revised, 284 pp., numerous microphotographs, \$18.00 (\$15.00 to members; \$12.00 to libraries). The American Academy of Ophthalmology and Otolaryngology, Medical Arts Bldg., Omaha, Neb.

Both these Atlases, developing from a syllabus to accompany a set of loan slides, are now so well known and have achieved so wide a distribution as to require little, if any, introduction.

Constructed on the loose-leaf plan, allowing easy amplification and revision, the left hand page carries the descriptive text which is illustrated on the right hand page by microphotographs of both high and low power.

The text is brief but to the point and written in an admirably clear style.

It is impossible to praise the illustrations too highly. Both Ray Mann Reeve, the photographer, and Miss Wilder, the Chief Technician who prepared the sections photographed, are clearly master craftsmen; and Captain DeCoursey and Lt. Col. Ash, who selected the fields for illustration, have the "seeing eye," as the results show.

These Atlases belong in every medical library, on the reference shelf of every

tissue diagnostician, and among the working texts of every specialist in these fields. Teachers of pathology, and particularly of special pathology, will welcome them for these atlases are outstanding in excellence.

A complete index enhances their practical value.

Laboratory Manual of The Massachusetts General Hospital. By Francis T. Hunter, M.D. Cloth, Ed. 3, 119 pp., \$1.75. Lea and Febiger, Philadelphia, Pa.

This little manual has, without doubt, been a life-saver for a host of internes in the past and the present edition, thoroughly revised and largely rewritten, will do as much for the internes of today and tomorrow.

Compact, concise and clear, its five chapters cover an amazing amount of ground and abound in practical, utilizable information. This book can be well recommended.

Anemia in Practice. By WILLIAM P. MURPHY, A.B., M.D., Associate in Medicine, Harvard Medical School, Senior Associate in Medicine, Peter Bent Brigham Hospital; Consultant Hematologist, Melrose Hospital, Melrose, Mass. Cloth, 344 pp., 40 figures, 5 colored plates. W. B. Saunders Co., Philadelphia, Pa.

This is a "must" item for the physician's library and may well be added to

the reference shelf of the clinical pathologist.

The work of Minot and Murphy in the development of the liver therapy of anemias is now a part of medical history. In this book Dr. Murphy presents this subject in an eminently clear and practical way, so much so that there can be, for the reader, no excuse for any clinical confusion in this matter.

The book is divided into two main sections, the first devoted to Hypochromic and Normocytic Anemias, the second, to Pernicious Anemia. Both sections are detailed and complete and are so written as to present clinical implications and applications in an excellently clear-cut manner.

This book cannot be too highly recommended as an eminently practical clinical text of especial interest and value to the practicing physician as well as to the clinical pathologist.

Principles of Hematology. By Russell L. Haden, M.A., M.D., Chief of the Medical Division of The Cleveland Clinic, Ohio; formerly Professor of Experimental Medicine in The University of Kansas School of Medicine, Kansas City, Kansas. Cloth, 348 pp., 155 illustrations, 1 colored plate, \$4.50. Lea and Febiger, Phila.

Dr. Haden is not only a hematologist of enviable repute but as Chief of a prominent Medical Clinic is eminently and peculiarly well equipped to write a book upon hematology of outstanding usefulness.

His method of approach is based upon two premises of essential importance: First, that "the disorders of the blood should be thought of as disturbances

in the normal physiology of the constituents of the blood instead of true diseases"; and, second, that "clinical hematology is simple if the fundamental principles upon which variations of the blood depend are thoroughly understood."

His book is written primarily for the physician and is, therefore, eminently practical in its method. Dr. Haden has the faculty of discussing difficult subjects simply and with admirable clarity. The inclusion of illustrative cases (100 in all) and of numerous original and admirable diagrams are outstanding features of the text. He has wisely left the unusual and difficult to those to whose fields they properly belong and presents only those phases of blood studies which fall within the purview of the practicing physician.

While it is true that there is practically no condition in which examinations of the blood may not reveal much of interest and practical utility, it is also true that such findings are not always suitably applied because they are not suitably appreciated. Dr. Haden's book bridges this gap between hematology per se and clinical medicine and as such deserves a place in the working library of every practicing physician.

It may be predicted with confidence that it will find such a place on its merits.

A good, well organized and well written book of practical utility.

A FURTHER SIMPLIFICATION OF THE KOLMER COMPLEMENT FIXATION TEST FOR SYPHILIS*

JOHN A. KOLMER

From the Department of Bacteriology and Immunology, Temple University School of Medicine, Philadelphia

For several years I have been greatly interested in a further simplification of my complement-fixation test for syphilis to meet the working conditions of Departments of Health and other laboratories required to conduct large numbers of examinations, and desiring to use a complement-fixation test with one or more of the flocculation procedures in the routine examination of sera and spinal fluids.

Without doubt, the serum diagnosis of syphilis is best served by using at least two tests routinely with every serum, as shown by the results of the serologic surveys conducted by the U. S. Public Health Service, and I believe that one should be a complement-fixation test by a method of proven sensitivity and specificity. This is particularly important because of the increasing number of States now legally requiring pre-marital tests and tests during pregnancy for syphilis, which carries a very heavy responsibility from the standpoint of sensitivity and especially specificity, since sooner or later an increasing incidence of difficulties may be expected from falsely positive reactions¹.

But in view of the constantly increasing number of tests required of State and municipal laboratories, the expense and time involved in the conduct of a complement-fixation test are matters of considerable importance. There is, however, no choice between a complement-fixation and any of the numerous flocculation procedures, insofar as training and experience of serologists are concerned. None are really technically simple and all require

^{*} Received for publication August 17, 1939.

great care and ample experience for their proper conduct to secure reactions of acceptable accuracy.

Insofar as my complement-fixation test is concerned, I still prefer and routinely employ the quantitative procedure using five amounts of serum and spinal fluid, but mainly because I believe the reactions are helpful as serologic guides in the treatment of syphilis, because prezone reactions, consisting of a falsely negative reaction with a large amount of serum (like 0.2 or 0.1 cc.) with a true positive reaction with smaller amounts (like 0.05 cc. or less), are very rare and practically do not occur at all with spinal fluids. From the standpoint of diagnosis, and especially when reactions are reported as positive, doubtful and negative, I have described a shorter or qualitative test employing two amounts of serum (0.2 and 0.1 cc.) and only one amount of cerebrospinal fluid (0.5 cc.) as the reactions are just as sensitive and specific as those observed with the quantitative procedure.

But I now believe that this qualitative test can be shortened or simplified by using only 0.2 cc. of serum. In other words, only two tubes are required for the test, the second being the serum control. Likewise in testing cerebrospinal fluid only a single dose of 0.5 cc. is required in each of two tubes, the second being the control.

Furthermore, while I routinely remove natural antisheep hemolysin from all sera before testing this is not essential, particularly when large numbers of tests are to be done, and its omission saves time. It is true that removal of antisheep hemolysin permits of a closer adjustment of the hemolytic system with a consequent increase of sensitivity but in the final analysis the incidence of reactions, insofar as positive, doubtful and negative are concerned, is not materially influenced, although syphilitic sera from which natural hemolysin is removed frequently yield stronger reactions.

For example, of 3000 tests giving positive Kolmer reactions with 0.2 and 0.1 cc. of serum in the Pennsylvania State Laboratories,* 38 or 1.3 per cent were stronger with the 0.2 cc. amount

^{*} I am greatly indebted to Dr. Verner Nisbit, Director of the Pennsylvania State Laboratories, for permission granted Miss Carola E. Richter to furnish these data.

of serum than with the 0.1 cc. amount. None were stronger with 0.1 cc. than with 0.2 cc. and none gave a positive reaction with 0.1 cc. and a negative reaction with 0.2 cc. In other words, prezone reactions were not observed and of the two amounts of serum, the 0.2 cc. dose gave the better results. Natural antisheep hemolysin was not removed.

In my laboratory, Mrs. Elsa R. Lynch has analyzed the last 2000 tests giving positive reactions with the quantitative test; natural antisheep hemolysin had been removed from the majority of sera. The results were as follows:

- (a) Two hundred and sixty-six or 13.3 per cent gave positive reactions with 0.2 cc. and negative with 0.1 cc. To the best of our knowledge all of these sera were from syphilitic individuals, many under treatment.
- (b) Four hundred and twenty-five or 21.2 per cent gave stronger positive reactions with 0.2 cc. than with 0.1 cc. and to the best of our knowledge all were from cases regarded clinically as syphilitic.
- (c) It is important that in no instance were positive reactions observed with 0.1 cc. and negative with 0.2 cc. in either the Pennsylvania State or my laboratory, totalling 5000 positive sera. In other words, prezone reactions did not occur, insofar as positive or negative reactions were concerned, although in my laboratory 13 or 0.65 per cent of the 2000 sera gave stronger reactions with 0.1 cc. than with 0.2 cc. of serum probably because of the removal of antisheep hemolysin. I believe, therefore, that the routine removal of natural antisheep hemolysin can be omitted when working conditions demand insofar as positive, doubtful and negative reactions are concerned, providing the tests are conducted with a 0.2 cc. amount of serum.

Under the conditions I recommend, therefore, that in those laboratories desiring to do the Kolmer complement fixation test, and where time and expense do not permit using the quantitative procedure, the tests be conducted with a single dose of serum (0.2 cc.) as follows:

1. Preliminary removal of natural antisheep hemolysin is not required.

^{2.} Heat the sera in a water bath at 55-56°C. for 30 minutes. In my regular test heating is for 15 to 20 minutes only and is preferred in order to reduce to a

minimum the destruction of antibody, but 30 minutes may be employed so that the sera are prepared in the single operation for the Eagle, Davies-Hinton, Kahn or Kline flocculation tests. Spinal fluids do not require heating unless kept for more than three days at room temperature (as during shipment in the mails) when heating at 55–56°C. is advisable for the removal of thermolabile anticomplementary substances.

- 3. For each serum arrange two test tubes and place 0.5 cc. of saline solution in the second or serum control tube.
 - 4. For each spinal fluid arrange two test tubes; no saline solution.
- 5. Place 0.2 cc. of serum or 0.5 cc. of spinal fluid in each of the two test tubes.
- 6. Add 0.5 cc. of Kolmer C.L. antigen, carrying the optimum dilution, in the first tube of each set.
- 7. Wait 10 minutes at room temperature when 1 cc. of complement (carrying two full units) is added to all tubes.
- 8. Primary incubation in a refrigerator at 6 to 8°C. for 15 to 18 hours followed by 10 to 15 minutes in a water bath at 37°C.
- 9. Add 0.5 cc. of hemolysin (carrying two units) and 0.5 cc. of 2 per cent suspension of washed sheep corpuscles to all tubes.
- 10. Secondary incubation in a water bath at 37°C. for one hour when the readings are made, although experienced serologists are advised to remove the racks and make readings 10 minutes after complete hemolysis of the serum, antigen and hemolytic system controls.

In other words, the technic is exactly the same as in the regular Kolmer test^{2, 3} insofar as the preparation and titration of hemolysin, complement and antigen are concerned as likewise the inclusion of antigen, hemolytic system and corpuscle controls. When large numbers of tests are conducted, controls with known positive and negative sera are not required but otherwise, and especially in the case of inexperienced serologists, they should be included.

I am convinced that a shorter primary incubation like 4 hours refrigerator or 2 hours refrigerator with $\frac{1}{2}$ or 1 hour water bath, etc., are not as satisfactory as that employed for securing reactions of maximum sensitivity consistent with specificity. Furthermore, the overnight incubation is a distinct advantage when large numbers of tests are being conducted. Certainly accuracy should never be sacrificed for mere speed since the rapid flocculation tests are ordinarily sufficient for pre-transfusion tests of the blood of donors. And I am convinced that adding

hemolysin and corpuscles separately, instead of combined together, is preferred because the reactions are more sensitive and since the difference in time involved is so small as to be almost negligible. Furthermore, the complement is delivered in 1 cc. amount while the antigen, hemolysin and corpuscles are delivered in 0.5 cc. each so that an inspection of the racks during the set-up readily detects any accidental omissions. And it is not necessary to add any saline solution at all except to the controls.

The test, therefore, is very economical in the time required as likewise with the reagents, since 5 cc. of guinea pig serum ordinarily furnishes sufficient complement for 100 to 125 tests while 1 cc. of antigen is sufficient for at least 1000 tests and 1 cc. of hemolysin for about 4000 tests.

The reactions may be reported as positive, doubtful or negative as recommended by the Committee on the Evaluation of Serodiagnostic Tests for Syphilis of the United States Public Health Service, cooperating with the American Society of Clinical Pathologists, as follows: Positive: +++++(4), ++++(3), +++(2) or +(1), in the first tube. Doubtful: \pm in the first tube. Negative: - in the first tube. However, I recommend reporting as strongly positive, weakly positive, doubtful and negative⁴ as follows: Strongly positive: ++++(4) or +++(3) in the first tube. Weakly positive: +++(2) or +(1) in the first tube. Doubtful: \pm in the first tube. Negative: - in the first tube.

Slightly anticomplementary reactions may be safely reported as follows: $4 \pm = \text{positive}$, $4 \cdot 1 = \text{positive}$, $4 \cdot 2 = \text{doubtful}$, $3 \cdot 1 = \text{doubtful}$, $3 \cdot 2 = \text{doubtful}$, $3 \cdot 3 = \text{negative}$, $2 \cdot 2 = \text{negative}$, $2 \cdot 1 = \text{negative}$, $1 \cdot 1 = \text{negative}$

With sera heavily contaminated with bacteria and those which are chylous or heavily tinged with hemoglobin from spontaneous hemolysis in which the presence of thermostabile anticomplementary substances is suspected, I recommend a modified Sachs Method for their preparation as follows:

- 1. Heat 0.5 cc. of serum at 55-56°C. in a water bath for 30 minutes.
- 2. Add 4.1 cc. of accurately titrated N/300 hydrochloric acid and mix.

- 3. After standing ½ hour at room temperature, centrifuge thoroughly and discard the sediment.
- 4. To the supernatant fluid add 0.4 cc. of 10 per cent sodium chloride solution. The acid is fixed by the precipitate of globulin; hence neutralization is unnecessary.
 - 5. This gives a 1:10 dilution of original serum ready for testing.
- 6. Place 1 cc. in each of two tubes and proceed as above described. In these tests the dose of diluted serum is equivalent to 0.1 instead of 0.2 cc. of undiluted serum. The reactions, however, are recorded and reported exactly as above described.

CONCLUSIONS

A further simplification of the Kolmer complement-fixation test for syphilis is described employing only one amount of serum (0.2 cc.) or spinal fluid (0.5 cc.) especially designed in the interests of time and economy for those laboratories conducting a large number of tests.

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THE BLOOD DONOR REGISTRY AS A SUBSTITUTE FOR THE BLOOD BANK*

C. A. PONS

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The knowledge that blood stored under refrigeration for a number of days can be administered with safety has created considerable interest, although opinion is divided as to its relative merits over freshly obtained blood in conditions for which blood transfusions are indicated. All authorities agree on its value to replace blood deficit resulting from severe hemorrhage and readily available blood may be life-saving, while to replace blood proteins in such conditions as nephrosis, nephritis with edema, etc., it is conceivable that refrigerated blood may be adequate.

It is apparent that greater supervision is needed over Blood Banks than is commonly believed necessary, if we are to maintain a wide margin of safety. In the hands of experienced workers the number of transfusion reactions has increased when refrigerated blood is used. Quantitative and qualitative studies have shown¹ a rapid diminution of granulocytes, platelets, fibringen, complement and immune bodies and Scudder² and his associates have demonstrated a daily increase of plasma potassium in preserved blood. None of the preservatives in use prevent the diffusion of potassium from the cells to the plasma and since parenteral administration of potassium is associated with toxic manifestations, it is probable that some of the transfusion reactions with stored blood are due to the increase of plasma potassium. This is particularly so when large amounts of stored blood are given in the treatment of severe hemorrhage. Fragmentation of the red cells and hemolysis are the most commonly recognized changes and this phenomenon alone has imposed a greater limitation of the storage period than formerly recommended. Some authori-

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ties believe six to eight days should be the maximum period of storage. Obviously there must be a rapid turn over of transfusions for the Blood Bank to be successful. For the foregoing reasons, it is impractical for the two hundred bed general hospital to establish a Blood Bank. I should like to question the advisability of storing blood for the less common blood groups, namely AB and B, and possibly for the subgroup A_2 , in the general hospital of four hundred beds.

Recognizing the fact that Blood Banks have made blood readily available, the Donor Registry is proposed to facilitate and speed up the selecting of donors in the smaller institutions.

Organization: A notebook is kept in the laboratory for each blood group, in which the donor's name, address and telephone number are recorded. From time to time the entries are passed to the files in the Donor Registry kept in the main hospital office. The Donor Registry should be in charge of a clerk specially trained in the fundamentals of blood grouping and cross matching. The transfusion clerk receives all requests for donors and makes all arrangements for typing, cross matching and transfusions. No requests or reports are made by telephone.

When a transfusion is decided upon, a written request is sent to the transfusion clerk.

The following blanks are used:

TRANSFUSION CLERK

	Date
that we plan to give him/her One (1) Se	of (Type
the necessary arrangements.	
(1	Signed)
Request for Typing and Cross Typing: Patient's Name	Laboratory Transfusion Report:
Blood TypeRoom	Date
	Patient's Name
Donor Type	Blood Type
	Donor's Name
	Blood Type
	Address

(Signature of Technician)

	Cross Agglutination is
Expected date of Transfusion	Kline Test on Donor is
	(Simpeture of Technician)

The following tests, which have so materially reduced the time required for the selection of a donor, are employed. These tests must fulfill two essential requirements, (a) specificity and (b) sensitivity.

 Defibrinated blood instead of serum is employed (estimated time saved— 10 to 15 minutes).

2. The Landsteiner Centrifuge Technic of cross typing is employed (5 minutes as compared to 30 minutes).

3. Inactivation for the Kline test is carried out at temperature of 60°C. for 4 minutes as compared to 30 minutes at 56°C.

4. Highly potent serum⁴ is used for typing.

How does the Donor Registry help? When a donor cannot readily be obtained from the relatives or friends, they are given the list of donors of the type required. From this list they may be able to approach donors. In this way the burden of obtaining donors rests with the patient's relatives, and the cooperative principle is maintained. In a comparatively short time a sizable Donor Registry can be compiled. During 1938, 3200 donors were typed at two hospitals with a combined capacity of 375 beds. Had we established a Donor Registry ten years ago, 30,000 individuals, or one-fifth of the population of our county, would have been catalogued.

By exchange with nearby institutions the donor lists can be increased. When donors are registered, we explain the purpose of registration, impressing upon them the importance of having available blood, and the difficulties encountered when one is limited to a few friends and relatives. The Blood Bank is explained, and the fact that, by a donor cooperative spirit, the small hospital can offer practically the same speedy service. It is a simple matter to develop community interest in this effort, and no public campaign is necessary.

While all hospitals have a professional donors' list, the Blood

Registry includes only non-professional donors. Patients able to pay for professional donors should be supplied with blood from that list.

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PROBLEMS IN BLOOD BANKING*

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In past years the charity patients at the John Gaston Hospital requiring blood transfusions were entirely dependent on volunteer donors because no funds were available for the payment of professional donors. Under this system many people bled to death or died in shock because compatible donors could not be obtained or could not be obtained soon enough, and because of the lack of blood many people were denied the benefits of surgery or their hospital stay was unnecessarily prolonged.

In April 1938, following the lead of the Cook County Hospital¹ and the Philadelphia General, a blood bank was started in Memphis to furnish blood for emergency cases and to utilize more advantageously available volunteer donors. At the beginning there was no special equipment and very little detailed information about how a bank should be run. In developing the bank from an idea to a working project numerous difficulties were encountered and many technical and administrative problems had to be solved which will here be discussed so that others contemplating on starting a bank may avoid some of our mistakes.

PERSONNEL

The blood bank has been operated under the guidance of the Resident Surgeon, the Senior Operating Room Nurse and the Director of the Clinical Laboratories, but the actual responsibility for the daily routine has been placed in the hands of a technician who is available only five afternoons per week. This technician types the bloods, attends to the collection of blood for culture and serologic tests, instructs the interns and nurses, traces reactions

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and does the bookkeeping. The cleaning of apparatus, the assembling of collection and administration sets and the assisting in the collection of blood are done by student nurses who shift at two week intervals. The typing of the blood of the recipient, the cross matching of the recipient's blood with the preserved blood, the performance of emergency serologic tests and the dispensing of blood are done by the rotating laboratory interns during their first two months of hospital service. The bloods are all collected by the ward interns and the transfusions given by them.

That the blood bank has been able to function as well as it has with such a continually shifting group and under so little senior supervision is due to the intelligent co-operation of the entire staff. It is obvious to every one, however, that many of our difficulties have been and will continue to be due to the relatively untrained staff.

It is our recommendation not to start a blood bank unless a full time technician or graduate nurse trained in serology and bacteriology is available, so that the responsibility for the project can be placed in the hands of *one* person. In addition, trained assistants must be provided so that the bank is open and ready for business at all hours of the day and night.

REFRIGERATION

The most essential and most expensive part of the equipment for blood preservation is a refrigerator. It is economy to purchase a standard model of the best grade, for the value of the blood stored in the refrigerator at any one time is greater than the cost of the refrigerator. If the refrigerator fails the blood has to be thrown away or given with fear of serious or fatal reaction.

When our bank started, a small domestic model electric refrigerator was used which proved to be entirely satisfactory in so far as blood preservation was concerned. During the time it was used there were less than 5 per cent reactions, none of them serious. The storage space within this refrigerator soon became inadequate and a 35 cu. foot, four door electric refrigerator was purchased. After this refrigerator was properly connected, adjusted and equipped with safety devices it proved to be satisfactory, but before this was done there were three failures in refrigeration, one due to the shutting off of electric current and two due to stopping of the motor. Because of our troubles with refrigeration the following precautions were taken and are recommended to others:

1. Inspection at three hour intervals by a member of the hospital engineer staff who is also to be responsible for the upkeep of the motor.

2. Place the refrigerator on an electric circuit separate from all other electric appliances.

3. Equip the refrigerator with warning signal lights which go on when the temperature within the box exceeds 6°C. or falls below 0°C. The lights are placed in the hall outside of the blood bank room where they can be seen by members of the staff continually passing by. The lights are connected to the same electric circuit as the hall lights.

4. Equip the refrigerator with a recording thermometer (fig. 1), so that the temperature within the box can be visualized at all times and, in case of failure in refrigeration, the duration of the failure and the degree to which the temperature rises can be accurately determined. The thermometer is operated by a spring mechanism so that it will function independently of the electric system.

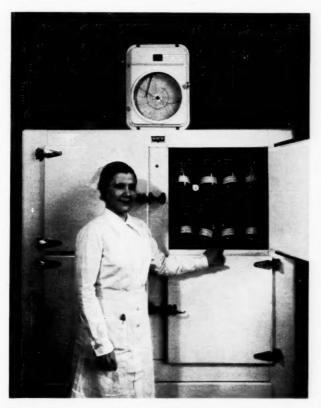


Fig. 1. Refrigerator with Recording Thermometer

Following refrigerator failure it has been our policy to discard all hemolized blood and to use the unhemolized blood for emergencies only. It has been shown in our series that blood can be warmed for a period of hours, rechilled and given at a later date without reactions, but it has also been shown that the use of such blood is associated with a higher percentage of reactions and more serious reactions than when the blood has been kept continuously cold.

Blood that has been frozen and later thawed is grossly hemolized. The freezing point of citrated blood lies between 0 and -1°C.

The optimum temperature to keep the blood is considered to be 4°C. Our refrigerator is adjusted to maintain a temperature range of 2 to 6°C.

The placing of warm bloods in the refrigerator and the continual opening of the doors for the purposes of blood typing elevate the temperature within the box and place an undue load on the refrigeration mechanism. It would be advisable to have a small subsidiary refrigerator in which the warm blood is placed and kept until the preliminary laboratory procedures are completed.

Those contemplating the starting of a blood bank might consider the possibility of having two or more medium sized refrigerators rather than one large one as being more safe and more economical to operate, provided that efficient use of storage space could be arranged. The safest arrangement of all would be to have electric and gas refrigerators so that in case of failure of one source of energy the entire amount of blood in storage would not be lost.

SEROLOGICAL TESTS

Because of the high incidence of syphilis among negroes and the inadequate treatment which they receive, preliminary Kline precipitation tests are performed by the laboratory interns on all negroes before they are selected as donors. After the blood for transfusion is collected in the bank, a check Kahn test is run in the serologic laboratory. White donors are questioned about primary and secondary lesions, blood tests and previous treatment, but preliminary tests for syphilis are not made before the bloods are taken. It has been found by experience that few bloods from white donors are discarded because of positive serology. The time saved to the donors and to the laboratory intern and nursing staff in not performing and keeping record of preliminary serologic tests is considerable and justifies the occasional discarding of blood.

CULTURE

When the bank was started the blood was collected by the open citrate method which had been in use in the hospital for years and was considered satisfactory. It was found that the blood cultures with this method were 100 per cent contaminated. A closed citrate method was then developed which for the first 6 months yielded 8 per cent positive cultures. During the next three months the percentage of positive cultures was reduced to 2.7 per cent.

The routine culture of all bloods became impossible with the technical staff available when the number of transfusions per month exceeded 150. The discontinuance of routine cultures was justified on the following points: 1. It was found that usually but few organisms were present and these mostly non-pathogenic. 2. The use of slightly contaminated blood was not necessarily followed by unfavorable results or reactions. 3. Bloods yielding positive cultures tend to become negative on standing in the refrigerator at 4°C. 4. The culture of blood from large flasks is technically difficult. 5. The removal

of the blood from the refrigerator for the purpose of culture causes the blood to warm up and interferes with its preservation.

Now, it is our policy to culture one blood each day which serves as a partial check on the sterility of our technique.

THE HEMOLYSIS TEST

When citrated blood is left standing in the refrigerator the hemolysis of red cells is retarded, but eventually the cells degenerate, the speed of cell degeneration varying in different bloods. In the majority of bloods there is no gross hemolysis during the first ten days. The detection of hemolysis by looking at the blood in a flask is unreliable, for it may appear to be hemolized when it



Fig. 2. Equipment for Hemolysis Test

is not or the supernatant fluid may appear normal although there is gross bemolysis in the cell layer.

In order to avoid the giving of hemolized bloods which are known to produce severe or fatal reactions, the bloods are routinely tested before they are given. The method used is to mix the blood in the flask thoroughly, remove 10 cc. by means of a sterile pipette, place the blood in a tube and centrifuge to separate the cells and plasma (fig. 2). If the supernatant fluid is definitely red the blood is considered to be hemolized and unfit for transfusion. If the degree of hemolysis is so slight that there is doubt as to the discoloration of the supernatant fluid, the rule has been to use the blood.

In the absence of routine cultures the hemolysis test serves as a rough test for gross bacterial contamination but cannot be depended upon as a test for sterility. Since the adoption of the hemolysis test as a routine in all bloods before they are given, the number of reactions has been greatly reduced.

BALANCE

During the first 12 months 1,905 flasks of blood were collected and stored in the refrigerator, and 1,415 transfusions were given. The capital in the bank at the end of the year consisted of 73 flasks of blood or 36.5 litres. During the year 417 flasks of blood or 21.8 per cent were discarded. The percentages of bloods discarded in the various blood groups were as follows: Type O, 22 per cent; Type A, 14 per cent; Type B, 25 per cent and Type AB, 68 per cent. The reasons for discarding the blood are summarized in table 1. It is seen that most of the blood that was thrown away was discarded because it was too old or because it was hemolized. Positive cultures, positive tests for syphilis, clotting and refrigerator failures account for smaller losses. Among the miscellaneous causes are overheating, failure to collect blood for serologic tests, breakage and technical difficulties in administration.

One of the reasons for having to discard so many flasks of blood is that we are forced by traditional prejudices to maintain separate stores of blood for

TABLE 1

REASON FOR DISCARDING	NUMBER]	PER CENT
Age (14 days +)	161	39
Hemolysis		22
+Culture	48	12
+Kahn	40	10
Clotting	30	7
Refrigerator failure	21	5
Miscellaneous	24	6

white and colored patients. Better co-operation is obtained from both groups by requiring each to provide blood for its own patients. The maintenance of two blood banks instead of one necessitates the storage of a larger supply of blood than can be efficiently used. There is no biologic reason for not using negro blood for white patients and vice versa. In emergencies the color line does not hold, for when a patient of either race needs blood, any available compatible blood is used.

Various methods of recording the bank balance have been tried. First the blood was credited to the service depositing the blood and blood given or discarded was charged against that service. It was found that certain interns were negligent in procuring donors so that the burden of maintaining balance fell unevenly on the more conscientious members of the house staff. Also, with a rotating intern service there was no incentive for interns to build up blood reserves, for blood collected on one service could not be used by a given intern on the next service. The system was changed so that now each intern has a separate account with the bank and is individually credited or charged

with all blood that he handles. If the intern has no credit his patients cannot receive blood. He can not borrow from the bank, but he can borrow from some other intern who has credit. If the blood is not replaced the intern who loans takes the loss. The intern's balance remains with him as he changes services, except that the blood taken for a given patient remains available for that patient as long as he is in the hospital. At the end of the internship whatever credit remains reverts to the intern who follows. Blood discarded for any reason is charged against the intern who collected the blood. This serves as an automatic brake against collecting more bloods than are necessary or being careless in collecting the blood. In order that the intern will maintain balance with individual patients, a special transfusion sheet has been devised which makes it possible to see at a glance how much blood a patient has to his cre iit and how much blood he has received.

It is always necessary to get more bloods than are to be given immediately, for the patient may need more than one transfusion and the blood may not be usable due to hemolysis or contamination. An attempt is made to get two donors for each contemplated transfusion, excess blood being used for patients with few or no friends and relatives. Patients who need blood immediately and have no credit in the bank receive it, but are expected to pay back as soon as donors can be obtained. The amount of blood in storage varies from 50 to 80 flasks. The optimum number of flasks is considered to be about 60. There has never been any difficulty in keeping a supply of blood on hand, although individual interns sometimes have difficulty, and sometimes there is an excess number of bloods of one type and a paucity of bloods of another.

The hospital pays nothing for the blood and makes no charges when the blood is given. The bank is a co-operative institution equipped and maintained by the hospital for the convenience of its patients, but the capital in the form of blood is furnished by the friends and relatives of the patients who are sick. By means of the blood bank and the exchange of blood of one type for another that is compatible the most expensive and most valuable of all therapeutic agents is made conveniently accessible to the indigent sick without imposing a burden on the tax-payer or on the hospital budget.

REACTIONS

After each transfusion a representative of the blood bank goes to the ward, studies the clinical record and from the temperature chart, nurse's notes, transfusion sheet and progress notes determines whether or not there has been a reaction. Any significant rise in temperature, with or without chill is interpreted as a reaction.

During the first 12 months reactions occurred in 6.7 per cent of the transfusions. The number of transfusions and the percentage of reactions according to months are given in figure 3.

During the year there were five deaths which occurred within a few hours

following transfusions. One was due to the use of mismatched blood. Another was thought to be due to blood which had been overheated. One death occurred at the time of refrigerator failure and was due to the use of hemolized blood. It was this death that caused us to adopt the centrifuge hemolysis test as a routine procedure. The fourth death occurred in a premature infant with syphilis and marasmus and was probably due to circulatory failure. The fifth death can be attributed to poor judgment in the use of transfusion since the patient receiving the blood had nephritis, azotemia and myocardial failure. There were no deaths of a delayed type with kidney failure and uremia.

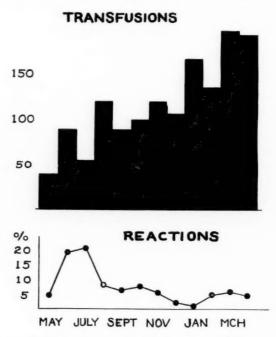


Fig. 3. Number of Transfusions and Percentage of Reactions According to Months

Analysis of the reactions according to the number of days the blood was stored in the refrigerator before it was given reveals that there is no appreciable difference in the percentage of reactions during the first 12 days, but that the giving of blood preserved longer than 12 days is associated with a significantly larger percentage of reactions (fig. 4). On the basis of these findings the rule has been made that blood will not be used after it is twelve days old.

Opinions differ as to whether it is advisable to heat the blood before use or whether it can be given cold. For the first 10 months we warmed all flasks of blood in a basin of warm water before use but the temperature of the water and the duration of warming varied widely and could not be controlled. On acquiring a constant temperature bath a controlled experiment was started in which the blood was on alternate weeks heated and not heated and the number of reactions noted. In heating the flask was placed in the water bath at 42°C. (fig. 5) and left for 15 minutes, during which time the temperature of the blood rose to about 30°C. During the weeks in which the blood was not heated it was taken out of the refrigerator, the test for hemolysis performed and the blood immediately filtered and sent down to the wards where it was

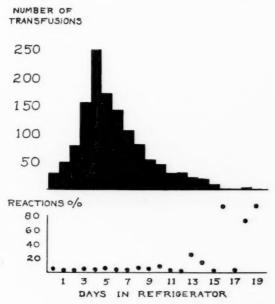


Fig. 4. Frequency Distribution of Number of Transfusions and Reaction Percentage According to Days in Refrigerator

given. During the five weeks in which the blood was warmed there were 219 transfusions with a reaction percentage of 7.3. During the five weeks in which the blood was not artificially warmed there were 206 transfusions with a reaction percentage of 6.3. The small number of observations and the numerous factors involved in the production of reactions do not warrant the drawing of final conclusions, but in so far as the experiment goes there is no evidence that advantage is gained by heating and no evidence that the giving of cold blood increases the number of reactions.

The heating of blood takes time and introduces an element of danger due to overheating. Patton² noted that "artificial heating has increased the in-

cidence of reactions." The warming of blood favors the growth of bacteria. On the basis of these combined facts it is our recommendation to give the blood without preliminary heating.

Blood banks will work effectively only in large charity hospitals in which five or more transfusions are given each day. The

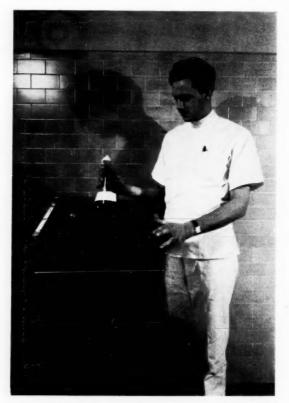


Fig. 5. Constant Temperature Bath

greater the number of transfusions and the more active the turnover of blood, the more efficient the bank becomes. When less than 100 transfusions are given each month it is difficult to maintain sufficient blood in storage to make compatible blood of all types available on demand without undue loss of blood due to aging. For this reason blood banks are not practical in small charity hospitals or in large hospitals with small charity services.

Blood banks will not work and are not needed in private hospitals, for individual doctors and patients who can afford to pay for blood and for services will insist upon having fresh blood at all times and will not co-operate in providing excess blood to cover inevitable losses.

In order that the bank may work most effectively all transfusions should be handled through the bank. When our bank first started some of the interns preferred to use fresh blood instead of preserved blood. This was allowed, but the services of the laboratory interns and technicians were not made available for those who did not choose to co-operate. As a consequence of this ruling the cross matching and the running of emergency serological tests on numerous donors for each transfusion ceased and few transfusions are now given using fresh blood.

In the light of recent investigations by Belenkiy³, Rhoades and Panzer⁴, Kolmer⁵ and others it is to be questioned if preserved blood is as effective as is fresh citrated blood in the control of hemorrhage in hemorrhagic diseases, and in the treatment of infections. If it is proved that preserved blood is less effective in the treatment of these conditions than is fresh blood then special arrangements can be made for special cases. One can always go back to the old volunteer system or use fresh placental blood.

Hemorrhagic diseases and infections constitute a very minor indication for transfusion in a general hospital. Most of the transfusions are used by the surgical and maternity services for the treatment of acute blood loss and shock and for pre- and post-operative treatment. In these conditions preserved blood has been proved to be effective and safe. Regardless of what may be proved concerning the inadequacies of preserved blood in special conditions, the blood bank is needed for surgical emergencies and as a means of making blood available to those who have no money and no friends or relatives. In charity hospitals like ours it is not a question of the relative merits of preserved citrated blood and fresh blood, but of preserved blood or no blood.

SUMMARY

Blood banks are needed in large charity hospitals to make possible the exchange of blood of one type for another blood that is compatible, make blood available for emergencies and provide blood for patients without friends or relatives. By means of a blood bank the indigent sick have access to ample supplies of blood without cost to the taxpayer or hospital, except for cost of administration and laboratory services. Blood banks will not work in small charity hospitals and are not needed in private hospitals.

In starting a blood bank the most essential things are:

1. A full time worker trained in surgical and in laboratory technique who is responsible for the entire project and for the training of assistants.

2. A refrigerator or refrigerators big enough to hold 60 or more flasks of blood, adjusted to maintain a temperature of 4°C. and equipped with adequate safety devices.

3. Facilities for performing essential laboratory tests such as typing, cross matching, culture, tests for syphilis and tests for hemolysis.

4. Equipment and facilities for taking and giving blood and for keeping records.

During the first year of operation of the blood bank at the John Gaston Hospital in Memphis 1,905 flasks of blood were collected, 1,415 transfusions given and 417 flasks of blood discarded. At the end of the year there were 73 flasks of blood in storage. The percentage of reactions, including elevations of temperature without chill, following transfusions was 6.7. There was no apparent increase in reactions with bloods stored for varying periods of time until the twelfth day. The number of reactions following the giving of blood that was not warmed and the giving of blood artificially warmed were essentially the same. There were five transfusion deaths.

Preserved blood has been proved to be effective in the treatment of acute blood loss and in shock. If it should be proved that the degenerative changes that occur on standing render preserved blood ineffective, or less effective than fresh blood in treating hemorrhagic diseases, infections and other special conditions, arrangements can be made for these conditions. In a charity hospital the majority of bloods are needed on the surgical and maternity services and here the question is not one concerning the relative effectiveness of fresh and preserved blood, but of preserved blood and no blood or blood after it is too late.

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PATHOLOGIC ANATOMY OF HUMAN BRUCELLOSIS*

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Bernhard Bang¹, in Denmark, in 1897 demonstrated the causal relation of the organism now known as *Brucella abortus* to the contagious abortion of cattle and called the organism *abortus bacillus*. In the western hemisphere the cattle disease designated as contagious abortion by practical farmers was shown by Mac-Neal and his co-workers² in 1910–1911 to be due to the organism described by Bang. They also amended the nomenclature to establish the specific name *abortus* for the microbe and cultures of this organism, isolated by them from cattle in Illinois, were utilized by Smith and Fabyan³, and by Fabyan⁴ in their studies of the pathology of this disease.

Although the experimental study of the pathologic anatomy of brucellosis was begun in this country, the major contributions in human brucellosis have come from abroad. Publications in English have not included illustrations of the granuloma believed to be characteristic of brucellosis; that deficiency and the recent publication of necropsy material⁵ prompt this report.

REPORT OF CASE

A 26 year old tractor operator was admitted to the New York Post-Graduate Hospital, December 14, 1937, complaining of unexplained intermittent fever. In May, 1937, while driving a tractor off a truck in a farmyard, the tractor turned over, knocking him to the ground and falling upon his left arm. The forearm was lacerated, and the humerus and radius fractured. Immediate debridement and reduction were done and a plaster spica applied. A week later broncho-pneumonia, verified by x-ray, developed and the spica was removed, to be reapplied 3 weeks after the accident. A month after the accident he was discharged as improved. While in the hospital he was seized

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by an attack of fever, marked perspiration and slight weakness, without chills or vomiting and between June and December, there were about 20 such attacks, the most recent lasting 3 days with a fever of 104.5°F. (40.3°C.). There was no loss of weight. On admission the left axillary lymph nodes were enlarged and the skin dusky, all wounds were healed, and temperature, pulse and respirations were normal. Following admission, however, the temperature progressively rose to 105.6°F. (40.9°C.) and the patient died with signs of cardiac failure, January 27, 1938, after a hospital stay of 45 days.

Soon after admission there was a positive macroscopic agglutination with Brucella abortus in dilutions through 1:320. Blood cultures were repeatedly sterile, until the day before death when Staphylococcus aureus was found. The Wassermann, Kahn, Widal and Felix-Weil tests were all negative. Brucella was not found in the feces or urine. There was a moderate anemia; on admission there were 3,750 white blood cells, 72 per cent of which were polymorphonuclear neutrophiles. When the anemia was most marked there were only 1,300 with unchanged neutrophile count, and 4,150 the day before death.

Necropsy: At necropsy, begun 6 hours after death, there was moderate pial edema and pulmonary congestion. The hilar nodes were not enlarged. There were no striking changes in the gastro-intestinal tract and mesenteric lymph nodes. The congested liver weighed 2300 grams.

The firm congested spleen, 890 grams, had a tense capsule, presented extensive areas of raspberry-red softening and the follicle markings were obscured. There was no evidence of suppuration in the left arm and forearm, with bony union well advanced.

Microscopic examination: There was nothing suggesting bacterial or rheumatic activity in the heart. The liver cells in the congested central areas stained poorly, had indefinite borders, and were disintegrating. In the liver capillaries there were small numbers of giant cells of irregular outline, 3 to 8 times larger than erythrocytes with neutrophilic homogeneous cytoplasm. The nuclei, with 2 to 8 overlapping lobes, were deeply basophilic and faintly vesiculated; some were peripherally arranged while a few were centrally placed. In some cells the nuclear chromatin was densely packed. In many cells only a narrow rim of cytoplasm was recognized because of the number and size of the nuclear lobes. None of these cells showed phagocytic activity and they were considered megakaryocytes.

Many of the portal fields were markedly enlarged at the expense of the surrounding liver tissue, the cells of which were atrophic, vacuolated and frayed. The bile ducts were unchanged and the blood vessels packed with erythrocytes and blood pigment granules. The stroma was proliferated, forming granulomas (fig. 1) with many cells of epithelioid type, having round to oval, palestaining vesiculated nuclei. The infiltrating lymphocytes were numerous and there were also polymorphonuclear leukocytes, both free in the tissues and in the capillaries. Giant cells up to 30, were the most striking feature and were

similar to those described in the sinusoids, but generally larger. Other giant cells had non-lobated nuclei, some were undergoing mitosis, and very few were of the Langhans type. The endothelium of the liver sinusoids was generally unaltered and the Kupffer cells were normally prominent. Centrally, in some lobules, the hepatic cords were necrotic and disintegrating but such areas were not extensive.

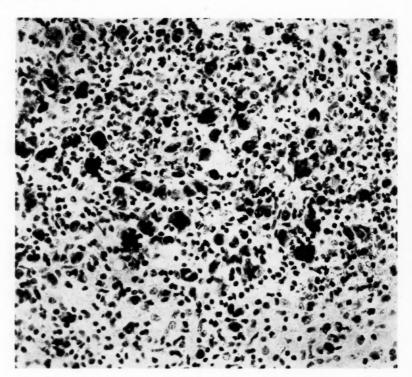


Fig. 1. Granuloma of Brucellosis in the Liver; \times 340

Most of the numerous giant cells strongly suggest enlarged megakaryocytes, while others are of epithelioid cell character.

The capsule and vessels of the spleen were unchanged except for one large vein with a well marked area of endophlebitis. In the intima, and lifting the endothelium, there was a cushion-like mass of proliferated stroma (fig. 2) formed chiefly of closely packed epithelioid cells. About one half was necrotic and suffused with red blood cells, and there was surrounding infiltration by lymphocytes. At the periphery of the granuloma there were two multinucleated giant cells similar to those described in the liver. The splenic follicles were greatly reduced in size and number, and many had giant cells similar to those

in the liver. Nuclei of other giant cells more closely resembled those of the few epithelioid cells also present. In the red pulp there were extensive confluent areas of necrosis, overrun by baked blood, blood pigment granules, scattered lymphocytes and giant cells. There were equally large areas of marked congestion and hemorrhage with many giant cells phagocyting brokendown blood pigment. There were smaller foci of partial necrosis with much free nuclear debris, polymorphonuclear neutrophiles and plasma cells in small numbers, as well as the epithelioid cells. Colonies of cocci were seen only in lumens of smaller vessels. Unfortunately lymph nodes were not preserved

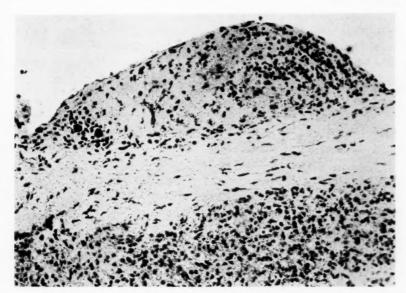


Fig. 2. Granuloma of Brucellosis in the Intima of a Vein within the Spleen; \times 158

The endothelium is lifted by the granuloma but is not injured

at necropsy. There were no significant changes in the pancreas, adrenals, or kidneys. At the sites of fracture there were changes similar to those in the liver and spleen and apparently not the result of the injury. There were large confluent areas of fibrinoid necrosis with both necrotic and non-necrotic phagocytes. These were large mononuclear cells, some with vacuolated cytoplasm, while others had ingested polymorphonuclear neutrophiles and lymphocytes. These apparently arose from the endothelium of the capillary spaces of the delicate fibrous marrow and from smaller areas of the cellular marrow in which there were numerous giant cells with lobated solid nuclei, apparently megakaryocytes, which were also present in thrombi in smaller arteries. Where the

fibrous marrow was more dense and mature there were non-necrotic periarteriolar granulomas formed of epithelioid giant cells similar to those in the liver and spleen, and lymphocytes. There were no striking changes in the brain or hypophysis, apart from vascular congestion. In the testis the tubules had narrowed lumens because of thickening of the lining epithelium. Spermatogenesis was incomplete and no spermatozoa were recognized.

In an attempt to find Brucella pieces of spleen were macerated and inoculated in liver infusion broth and in dextrose. Half the cultures were incubated in an atmosphere reinforced with carbon dioxide. All cultures yielded *Staphylococcus aureus* and were negative for Brucella. Some of the macerated material was injected subcutaneously into a pregnant guinea pig that died within three days without aborting. Brucellae were not recovered from its placenta, material from which was injected into a second pregnant pig with a similar fatality and negative bacterial results.

DISCUSSION

No attempt is made to report material already covered by Sharp⁶ in his exhaustive review or by Sprunt and McBryde⁷, except where difference of opinion exists. Publications that may have been overlooked, as well as those since printed are included. There is no apparent difference between the melitensis and abortus types of Brucella in ability to produce disease in the experimental animal. In man there are, unfortunately, relatively few necropsy reports in which the infecting organism is named⁷⁻¹². The human subject is infected by the handling of aborting cattle^{5, 9c, 13, 14}. My patient was injured while working in a farmyard while unidentified injury is also reported 106. Drinking raw cow's milk is held responsible 10b. 15, as well as goat's milk^{8e}. In one case¹⁶, a child played in a stable with, and drank raw milk from aborting cows. Brucellosis is commonly a disease of adults and is rarely reported in children 7. 16. 17. This has been attributed to several causes: the child may have natural immunity; he drinks pasteurized milk; he does not handle farm animals; his sensitivity has not been heightened by other diseases; the agglutination test is rarely done on children; there may be inadequate agglutinins¹⁷.

Lesions in experimental animals resemble those seen in human subjects. Fabyan⁴ reported a chronic inflammatory focal lesion, largely perivascular, very much like that of tuberculosis and

composed of epithelioid cells, with few lymphocytes, isolated plasma and giant cells; mitoses were numerous. Focal necrosis, common in the livers of some animals, was rarely seen in the lymph nodes. A decade later Jaffé¹⁸ repeated Fabyan's work, producing similar lesions after 6 to 8 weeks. The lesions differed from tubercles in that the epithelioid cells were less compactly arranged, stained less deeply, and were better defined, and there was no central caseation. Phagocyted bacilli were recognized. After 3 to 4 months the granuloma sites were marked by loose, poorly cellular connective tissue. After intratesticular injection there were also nodules in the enlarged spleen and lymph nodes composed of epithelioid cells, or, as Jaffé called them, "pale cells," together with a few megakaryocytes. The liver and kidneys had fewer nodules.

Experimental work has also been reported by Klimmer and Haupt¹⁹, Matzdorf^{10a}, Neberle and Pallaske²⁰, and most recently by Aiello²¹, Rothmann^{9c}, and Signorelli²². Depending on the allergic state Aiello²¹ produced either necrotic hemorrhagic lesions in a state of septicemia, or proliferation of the reticuloendothelium followed by the formation of granulomas which he believed specific for brucellosis. These were composed of epithelioid cells, eosinophiles, a few monocytes, polymorphonuclear neutrophiles and pseudo-Langhans giant cells. In some of the animals of Signorelli²² the granulomas in bone marrow and extramedullary foci had many giant cells of "polykariocytic and megakaryocytic" type. Almost identical results were obtained with live and inactivated bacteria. The megakaryocytic type of giant cell was phagocytic in the spleen, illustrations of which look like the lesions in the case reported here. Rothmann 9c injected guinea pigs with bacilli cultured from the organs and blood of his human case. After 8 weeks there were epithelioid cell nodules in the lungs, liver and spleen with giant cells having 20 and more nuclei; lymphocytes and plasma cells formed the periphery. In the larger nodules there was necrosis and fibrin deposit. The nodules in the animals were larger, with more giant cells, more connective tissue, and a greater tendency to necrosis than in humans. Rothmann thought the granuloma specific for brucellosis.

In human brucellosis the anatomic findings have been less constant, probably due to numerous varying and uncontrolled factors of allergy, emphasized by Rössle²³ in both this disease and typhoid fever, immunity, dosage and virulence of the microorganisms. No definite pathologic anatomic changes are reported in one case¹⁵, while many note no distinctive or significant alterations 8a. 14. 24-27. In one such case 8a there were circumscribed foci of connective tissue, as well as areas of necrosis in the spleen. The former may have represented healed lesions and the latter the response to terminal anergy. Uncharacteristic anatomic changes, similar to those in many other diseases, have been produced in the experimental animal by the intraperitoneal injection of tubercle bacilli as well as India ink²⁸. Only after a week did epithelioid cell tubercles appear in the lymph nodes. Similar findings were reported in typhoid fever and were likened to those in brucellosis²⁹. In human brucellosis von Albertini and Lieberherr⁵, who referred to the granuloma as a "tuberculoid nodule," were of the opinion that "... certain cases of Bang's disease can lead to the picture of a granulomatous affection. In contrast to tuberculosis, however, these cases form the exception." Allergy, dosage and time intervals apparently play the major rôles in the production of the granuloma.

Another feature of the anatomic picture that excites interest is the giant cell. In many cases the giant cell is of the Langhans type^{5.8d.21.30}, but a few authors^{9c.16} call attention to the megakaryocytic character of the cell. Megakaryocytes are not uncommon in vessels in diseases with much bone marrow stress. This exists in brucellosis where destruction and phagocytosis of red cells is a prominent feature. The megakaryocytic giant cells in the granulomas described in this paper were generally larger than the megakaryocytes in the vessels and were definitely multilobated. In one case³⁰ the giant cells were described as similar to Sternberg giant cells of lymphogranulomatosis. Multinucleated epithelioid cells similar to those also found in my case have been reported^{9c}.

Changes have been reported in virtually every organ in the body. Bergmark³¹ found a chronic meningoencephalitis with

round cell infiltration, especially about some vessels, in the pons and about the Sylvian fissure. Regressive changes were seen in large ganglion cells, in part with disintegration. Brucella was recovered from the spinal fluid in another case³² where the meninges were studded with "tubercles," formed chiefly of hyalinized connective tissue with chronic inflammatory cells. Earlier stages, including necrosis were seen. A meningoencephalitis comparable to that in typhus has been reported¹⁴. Throughout the nervous system there were nodules of round cells, plasma cells, leukocytes and especially glial cells. Brunner^{11b} reported an abscess in the thyroid with *Brucella abortus* in pure culture, which was also in the blood. Non-specific diffuse fibrosis of the thyroid with "histioid" cells in areas of lymphocytic infiltration has been described³⁰. Characteristic lung lesions have been reported only in experimental animals^{9c, 20}.

Brucella melitensis was recovered from the blood and from a thrombus on the heart valves^{8b, 8c, 9c, 10c}. Negative blood culture and tissue bacterial findings in a case with marked aortic ulcerative thromboendocarditis are reported¹⁴, while in another^{8t} only the culture was positive. In a child there were foci of lymphocytes, a few plasma cells and large cells with kidney-shaped nuclei about the epicardial vessels and diffuse lymphocytic infiltration of the myocardium¹⁶. "Interstitial myocarditis" was reported in other cases^{5, 10a}. Rothmann^{9c} saw subendocardial foci of necrosis surrounded by lymphocytes, epithelioid cells and plasma cells; media and intima of myocardial vessels were necrotic and there were bacteria and fibrin in the lumen. Vascular lesions include a myocotic aneurysm of a cerebral vessel with rupture³².

The granulomas believed to be characteristic of brucellosis are most often seen in liver^{9b, 34, 35}, spleen^{8b, 30, 36}, or in both^{5, 9c, 13, 16, 37-39}, as in the case reported here. Necrosis of the granulomas has been described, chiefly in those without or with but poor formation of giant cells⁵. Specific and non-specific alteration were seen in the same organ^{9c, 37-39}. Gall bladder and liver lesions were described by Mettier and Kerr^{9b} whose paper has the only illustrations of Brucellar granulomas encountered in the English or American literature. The granulomas pictured

are in a fibrotic stage, however, and fail to reproduce adequately the more characteristic features. Loffler and von Albertini¹³ called attention to splenic endophlebitis and thought it produced involvement of the liver through the portal vein, and that brucellosis is therefore chiefly a hepatolienal disease. The infrequency of vein lesions³⁶⁻³⁸ and the virtually obligate splenomegaly prompted von Albertini and Lieberherr⁵ later to attribute the major rôle in brucellosis to the spleen. They thought that each attack of fever is produced by an invasion of Brucellae into the blood stream; the organisms come from the spleen and are removed from the blood by the "so-called primary blood filters with their reticuloendothelial apparatus." The absence of characteristic anatomic changes at many necropsies was explained by assuming that such cases are pure bacteremic forms, that the reticuloendothelial apparatus of the filters overcomes the infection, and that as a result granulomas are not formed; hence, the infrequency of granulomas.

Lymph node granulomas were seen in most of the cases with and in some without splenic lesions 9a, 33. The diagnostic problems so commonly presented by lymph node changes are well illustrated by Grumbach's case¹². His patient, with a positive agglutination (1:640), had a periostitis of the tibia and of a malleolus, and after the lesions softened, Brucella abortus, porcine type was recovered. A similar organism was found in pus from a lymph node. A lymph node, removed at biopsy, was diagnosed as tuberculous by von Albertini⁴⁰, although no tubercle bacilli were seen in the sections and no animal inoculation was done. marrow lesions, as described in my case and similar to those in other organs, have been reported e. 39. 38. Sprunt and McBryde 7 called attention to the replacement of normal marrow cells by atypical mononuclear cells, findings generally similar to those of Eyre⁴¹, while foci of "histioid reaction" in the marrow appear in another report30.

The only reference to the adrenals recorded bacteria in the vessels and areas of necrosis^{9c}. In kidneys, granulomas, some with necrosis, have been described^{5.8d.13.30.35} while acute glomerulonephritis was seen by others^{10a.16.33}, once with granulomas^{9c}.

Non-specific changes are usually not recorded. Apart from the few observations reviewed by Sharp⁶, there are only isolated reports of changes in the genital tract. Wegener³⁷, who is the only author describing granulomas in the salivary glands, reported similar lesions in the testis interstitium, and large foci of necrosis in prostate and seminal vesicles. Amoss⁴² described the unique case of a woman in whom, at operation, the right oviduct was seen to have lesions resembling those of tuberculosis. From the same oviduct Brucellae were recovered. Two acid-fast bacilli were also seen in several sections of the same organ and material from the tube produced tuberculosis in a guinea pig. Brucellae were also found in small cysts in the ovary, in adjacent lymph nodes, in the appendix, ileocecal lymph nodes and in the lesions of the peritoneum which looked like those of tuberculous peritonitis. The granulomas, histologically, were like tubercles with giant cell and lymphocyte infiltration, but there was less connective tissue than is usually seen in tubercles.

It seems not unlikely, when one considers the relatively few fatalities in brucellosis, that the body is able to destroy the invader. No specific evidence of the invasion is left behind since relatively few necropsies show specific anatomic evidence of the disease. Those patients who die early in the disease show the features that are common to all malignant bacterial diseases. Only the patients in whom the equilibrium of attack and defense continues for months develop the lesion that may be regarded as characteristic. Combined with clinical evidence of brucellosis, the lesion can probably be considered specific.

SUMMARY

A 28 year old tractor operator sustained compound fractures of the arm while working in a farmyard. Soon afterward, and continuing until death eight months later, there were repeated attacks of fever associated with anemia and leukopenia. The macroscopic agglutination test with *Brucella abortus* was positive in dilutions through 1:320. At necropsy there was general visceral congestion and evidence of continued breakdown of red cells. The bone marrow, enlarged liver and spleen, and the splenic

vein were sites of granulomas. These consisted of epithelioid cells, lymphocytes and giant cells, many of which were multilobated and resembled enlarged megakaryocytes. The granulomas were similar to those described in the literature, which is reviewed. The relative rarity of findings of granulomas is expained by the comparative case of the body to finally overcome Brucellar infection. Only in protracted illness is the characteristic granuloma formed.

ADDENDUM

Since this work was completed, additional references have been found. Three cases of blood stream infection are reported, one with aortic⁴³ and two with mitral endocarditis^{44, 45}. Nicolaewa⁴⁶ described eye changes similar to those seen in syphilis and tuberculosis. One recent report⁴⁷ describes "small nodular lesions" in lungs, liver bone marrow, spleen and lymph nodes, while another⁴⁸ records granulomas in liver and spleen.

Thanks are due Dr. Walter E. Lough for permission to use the clinical records and to Dr. Henry Weinberg, assistant Medical Examiner, City of New York, under whose direction the necropsy was performed. Miss Martha J. Spence did the bacteriological studies, Miss Alice E. Slavkin gave technical assistance, and Miss Doris Richey made the photomicrographs.

REFERENCES

Owing to their number, references are not printed but will be included in the author's reprints.

CARCINOMA OF ESOPHAGUS PERFORATING THE AORTA*

HERBERT J. SCHATTENBERG AND JOSEPH ZISKIND

From the Department of Pathology, Graduate School, Tulane University, and the Charity Hospital, New Orleans

Perforation of a carcinomatous ulcer of the esophagus is not Kaufman¹ found that it occurred in 45 per cent of uncommon. his cases. The perforation may take place into the trachea and bronchi, the mediastinum, large blood vessels and pleural or pericardial cavities. Erosion of large blood vessels with fatal hemorrhage, is, however, quite rare. Kaufman¹ noted erosion into the aorta four times in 126 cases of carcinoma of the esophagus. Knaut² collected all reported cases up to 1896 which numbered 50, and since Knaut's study Carr and Hanford³ in 1922 found 21 cases in the literature and added one of their own. Since 1922 occasional cases have been added to the literature. We have recently had a case of esophageal carcinoma which perforated into the aorta and caused fatal hemorrhage and because of the rarity of this condition and the long history of esophageal bleeding we believe this case is of sufficient interest to warrant reporting.

REPORT OF CASE

H. R., a colored male, 45 years of age was perfectly well until January 1937 when he suddenly became hoarse, and in spite of symptomatic treatment, this condition never improved. Since the onset of hoarseness he noticed some difficulty in swallowing food. On July 3, 1937, five hours before admission to the hospital, he suddenly experienced a severe pain in the chest and abdomen following which he bled from the oral cavity. The patient estimated that he lost approximately one quart of blood.

Physical examination revealed a well developed and nourished colored male about 45 years of age, who was restless and perspiring. The B. P. was 110/70;

^{*} Received for publication April 4, 1938.

the temperature 100; the pulse 120 and the respiration 24. The pupils were equal, regular and reacted to light and accommodation. The nose, ears and throat were negative. The neck showed no abnormal or visible pulsations, masses or adenopathy. The lungs were clear. The heart was not enlarged, the rate was rapid and no murmurs were heard. The aortic transverse diameter



Fig. 1. View of Descending Aorta with Probe in the Perforation

appeared to be widened. The abdomen was tender in the umbilical region. No masses or abdominal organs were palpable. The extremities were normal. Laryngoscopy revealed paralysis of the left vocal cord. The Blood Wassermann was negative. The hemoglobin was 70 per cent and the white cell count was 11,250. The urine examination was normal. X-ray examination of the chest showed no significant increase in the perihilar and peribronchial markings and

no evident lesion in the lung parenchyma. There was some increase in width of the upper mediastinal shadow suggesting dilatation of the aorta. The patient was put to bed and treated with sedatives. His temperature, pulse and respiration gradually came down to normal and he left the hospital on July 12, 1937. The patient was readmitted on September 15, 1937, with the following



Fig. 2. Ulcerating Carcinoma of Esophagus Demarcated by Dotted Line.
Probe in the Central Necrotic Area

history. He felt fairly well since his discharge until four days before this second admission when he developed persistent vomiting. Shortly before admission he had a severe oral hemorrhage, which continued while in the hospital and caused the patient's death three hours after arrival on the ward.

Autopsy. The body was that of an emaciated, poorly developed colored

male, about 45 years of age, weighing approximately 130 pounds and measuring 165 cm. in length. The conjunctivae and sclerae were very pale. The oral cavity was filled with many blood clots. The rest of the external examination was normal. The pleural cavities were partially obliterated by adhesions.

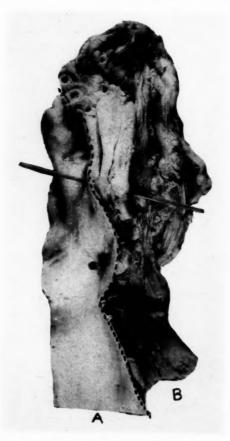


Fig. 3. View Showing Path of Perforation as Indicated by Probe Dotted line demarcates agrta from esophagus. (A) indicates agrta and (B) esophagus. Esophageal wall markedly thickened by tumor.

The lungs showed moderate emphysema and the bronchi were normal. The heart weighed 300 grams and the myocardium was flabby. The coronary arteries were patent. At the junction of the transverse and descending aorta a perforation was noted in the intima (fig. 1) which communicated with the lumen of the esophagus adjacent to it. The rest of the aorta showed a moderate

amount of arteriosclerosis but none at or just surrounding the perforation. In the middle third of the esophagus in the region of the bifurcation of the trachea, a tumor mass (fig. 2) was noted which measured 8 cm. by 4 cm. by 0.5 cm. and almost completely occluded and surrounded the esophageal lumen. The wall



Fig. 4. Groups of Squamous Cells Not Well Differentiated

of the esophagus was thickened by the tumor mass. Within the center of the tumor, an area was noted measuring 1.5 cm. in diameter which was very soft and necrotic and a probe could be passed through it directly into the aorta through the previously described perforation (fig. 3). The aorta and esophagus were adherent to each other at this level for a distance of about 4 cm. The

regional esophageal lymph nodes, the bronchial and the posterior mediastinal lymph nodes were all considerably enlarged, firm and involved with neoplastic tissue. The stomach was distended and filled with an enormous blood clot. The small and large intestines contained altered blood. The liver, lungs, kidney, spleen, gastro-intestinal tract, heart, adrenals, pancreas, and genital organs showed no evidence of any metastatic involvement.

Microscopic findings. The tumor of the esophagus was composed of groups of squamous cells whose centers showed, in a few instances, some slight attempt at cornification. In general, it was considerably undifferentiated (fig. 4). Considerable necrosis and secondary infection was present. The regional lymph nodes were infiltrated with similar groups of neoplastic cells. The tumor was classified as a squamous cell carcinoma—Grade III.

DISCUSSION

Carcinoma of the esophagus begins by an infiltration of the mucosal cancer cells into the deeper layers of the esophageal wall. The tumor grows between the muscle fibers to the adventitia and together with the reacting connective tissue causes thickening of the esophagus. The normal layers are forced apart and destroyed and the wall is then composed entirely of neoplastic and fibrous tissues.

Perforation of the aorta with fatal hemorrhage is one of the rare terminal complications of this disease. In ulcerative carcinoma of the esophagus with associated infiltration of the aorta, perforation will usually occur as the tumor disintegrates before the advancing ulcerative process. In some cases perforation is direct because of suppuration of the tumor.

The case herein reported shows a tumor of a high degree of malignancy with metastases to the regional lymph nodes. This is in line with the findings of Vinson⁴ who studied and reported his findings in 1000 cases of malignant disease of the esophagus and concluded that metastases are frequent and undoubtedly occur early in many cases. He takes exception to the prevalent view that carcinoma of the esophagus is a slow growing tumor of low grade malignancy with very little tendency to metastasize. This observer noted in those 519 of his cases which had tissue removed for examination that many of these were of a grade 3 or 4 according to Broder's classification. Vinson concludes, further,

that esophageal carcinoma is usually of a high degree of malignancy, with little cell differentiation and with frequent metastases.

SUMMARY

A case of carcinoma of the esophagus with perforation into the aorta is reported.

Metastases to the regional lymph nodes were present and the tumor was of a high grade of malignancy.

Perforation of the aorta from an esophageal carcinoma with fatal hemorrhage is a rare complication of this disease.

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CORRESPONDENCE

To The Editor:

In the July 1939 issue of the American Journal of Clinical Pathology appeared an article by I. Davidsohn and I. Rosenfeld dealing with "The Preparation of Anti-M and Anti-N Testing Fluids." In describing the technic of preparing immune anti-M and anti-N sera the results by two different methods were compared, one of the methods being attributed to Levine, the other to myself.

The method attributed to me was referred to as a "rapid method," and is described as follows:

"Daily intravenous injections of washed red blood cells of type OM or ON beginning with 0.1 cc. and increasing by 0.1 cc. until the dose of 0.5 cc. is reached. Seven days after the fifth injection the rabbits are bled from the ear vein, the blood serum is absorbed with a mixture of blood cells of types OM, A_1M , and BM or ON, A_1N , and BN, to determine the suitability of the serum for preparation of testing fluids. If the serum is found satisfactory, the rabbit is exanguinated. Usually several courses of immunization were necessary. The intervals were one or more months. This detail differed from the original recommendation of Wiener of seven days' rest periods between each course of injections."

As a matter of fact, the present writer has never described any "rapid" technic for preparing immune sera against M and N. In fact, in my experience, at least three courses of injections, and at times four or more, are required before a good immune serum is obtained. Therefore it is my practice not to test the potency of the rabbit serum until 5 to 7 days after the last injection of the third course, that is, no sooner than six weeks after the first injection is given. Such a practice would hardly be called a "rapid" technic, and I am not at all surprised that Davidsohn and Rosenfeld obtained poor results after only a single course of injections.

In order that the technic ascribed to me by Davidsohn and Rosenfeld may be compared with the technic as it is actually given in the following references (Wiener, A. S.: Blood Groups and Blood Transfusion, p. 120, C. C. Thomas, 1935; Wiener, A. S.: Amer. Jour. Med. Sci., 186: 257, 1933; Wiener, A. S., Zinsher, R. and Selkowe, J.: Jour. Immunol. 27: 431, 1934), I shall quote from the first named article the technic as outlined there:

"The technic I have found most effective is to alternate courses of daily intravenous injections with long rest periods. If 12 rabbits are to be immunized, 25 cc. of blood will suffice for one course. The blood should be divided among

7 or more tubes (for a course of 7 days or longer), containing the following solutions, recommended by Rous and Turner for preserving blood: 5.4 per cent glucose (5 parts), 3.8 per cent sodium citrate (2 parts); whole blood (3 parts).

"The solutions must be sterile, and the blood must be collected under sterile precautions. When stored in the ice box in this manner, blood may be kept for several weeks. Before injecting the blood into the rabbits, the contents of each tube should be washed with sterile saline and then diluted up to a convenient volume. The washed blood is then divided equally among 12 rabbits. All the injections of the first course are given intravenously. The first injection of each of the subsequent courses is given intraperitoneally (to avoid anaphylaxis); all other injections are given intravenously. The rest periods between the courses should be about 7 to 10 days. The rabbits are bled and the sera examined for their agglutinin content 1 week after the last injection. After 3 or 4 courses of injections, most rabbits will produce good anti-N sera. It is much more difficult to produce anti-M sera, however. We finally succeeded in producing a very potent anti-M serum, by giving 2 rabbits which had had several courses of injections, followed by a rest period of several months, 1 additional course of injections. When preparing these sera it is wise to start with a large series of rabbits, since because of the protracted nature of the immunization (particularly for M), most of the rabbits will die before the experiment is completed."

It will be seen that the above outline emphasizes the "protracted nature" of the immunization, so that it could hardly be called a "rapid" technic.

Another minor difference in the technic as described by Davidsohn and Rosenfeld, and as actually given by myself, is that the amount of blood given at each injection of the course, is the same, and does not increase day by day.

Finally, it might be mentioned that the use of intervals of one or more months is not novel, since the advantage of long rest periods is emphasized in the present writer's description of the technic—in fact, as indicated above, in the original article appears the phrase "rest period of several months," the phrase "several months" being italicized.

Incidentally, in recommending the technic given above for the preparation of anti-M and anti-N sera it was not the intention of the present writer to claim any great originality, since to my recollection similar procedures have been used for the preparation of other immune sera. To cite, for example, from my own experience, it might be mentioned that the method has been used with success for the preparation of precipitin sera and sheep cell amboceptor.

Very truly yours,

A. S. WIENER, M.D.

To The Editor:

The technic of immunization of rabbits, which we designated in our article (page 400) as Wiener's method, was copied by us from Wiener's publication

(Am. J. Med. Sc., 186: 257, 1933). We stated in our article: "Usually, several courses of immunization were necessary. The intervals were one or more months. This detail differed from the original recommendation of Wiener of seven days' rest period between each course of injections." In the text of Wiener's publications, as quoted in the preceding letter, it is stated: "The rest periods between the courses should be about seven to ten days." The phrase: "The rest period of several months" which occurs later in the text, seemed to us to be recommended not as a routine procedure but in cases when the routine method of immunization will fail, as in the quoted instance of the two rabbits. We recommended the longer intervals of one or more months as a regular procedure, and therefore we felt obligated to point it out as a deviation from the technic as recommended in Wiener's publications. Wiener objects to the adjective "rapid" which is applied to his technic. The term "rapid" method was used instead of "Wiener's" method merely for the sake of brevity and convenience. We found that occasionally after a single course of five daily intravenous injections, a good serum was obtained (see Rabbit No. 20 in table 2). In terms of days that means obtaining a serum in twelve days instead of twentyseven, which is the shortest period that immunization according to Levine's method can be completed. It is for that reason that we applied the adjective "rapid" to Wiener's method.

It was not our intention to convey the impression that Dr. Wiener advocates a "rapid" method.

Very truly yours,

I. Davidsohn.

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